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VOLUME 28

PUBLISHED BI-MONTHLY FOR
THE AMERICAN CHEMICAL SOCIETY
BY
THE WILLIAMS & WILKINS COMPANY
Baltimore, U. S. A.
1941

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ERRATA

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Page 343: It should be pointed out that, although Cope (18) used salicylic acid in the determination of nitro nitrogen, this substance was first suggested and used by Professor M. A. Scovell (pages 51-54, *Methods of Analysis of the Association of Official Agricultural Chemists*, 1887, Bulletin 16, Division of Chemistry, United States Department of Agriculture) for the analysis of nitrogen in nitrates.

Page 347: In references 37 and 48, read "1889" for "1899".

REACTIONS OF HYDROCARBONS IN ELECTRICAL DISCHARGES¹

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Received August 6, 1940

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I. INTRODUCTION

The reactions of hydrocarbons in electrical discharges are interesting and important from both the practical and the theoretical points of view. Theoretical studies establish what reactions occur and throw light on the mechanism of activation which causes reactions at room temperature. If the theory of these reactions is known, it is possible to utilize them to the greatest advantage from the practical point of view.

The present study seeks to establish the fundamental chemistry of the hydrocarbon reactions occurring in these discharges and at the same time to present some mechanisms and theory of these reactions. All of the information that would be desirable for such an evaluation is not available, since complete experiments have not been made.

¹ Presented before the Division of Petroleum Chemistry at the Ninety-fifth Meeting of the American Chemical Society, held in Dallas, Texas, April, 1938. The references have been extended to May, 1940.

II. TYPES OF ELECTRICAL DISCHARGE

Broadly, electrical discharges can be divided into two main classifications. (A) non-disruptive or silent discharges and (B) disruptive discharges. These may be further subdivided as follows:

A. Non-disruptive or silent discharges:

- 1 The ozonizer discharge
- 2 The semi-corona discharge
- 3 The corona discharge
- 4 The glow discharge
5. The electrodeless discharge

B Disruptive discharges

- 1 The spark
2. The arc

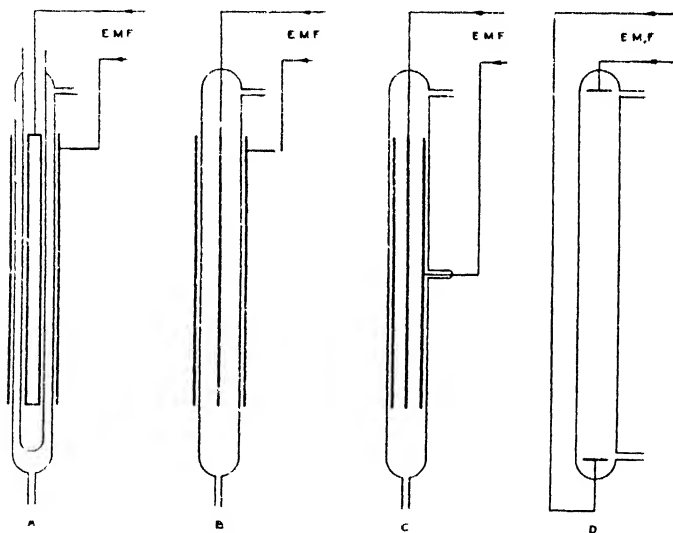


FIG. 1 Types of discharge apparatus. A, ozonizer discharge; B, semi-corona discharge, C, corona discharge; D, glow discharge.

The type of discharge used in studying any particular hydrocarbon reaction often plays a very important rôle in determining the reactions which occur and the products obtained. The disruptive discharges are accompanied by a localized high-temperature zone in and near the discharge, so that the reactions are a combination of the thermal reactions due to this high temperature and the electrical reactions from electrons present and the electrical field. The silent discharges are more diffuse and are not accompanied by such high-temperature zones. The silent discharges therefore cause chemical reactions that are due, almost entirely,

to the electric field. For this reason the reactions in the silent discharges will be presented first.

The types of silent discharge may be differentiated by referring to figure 1.

A. NON-DISRUPTIVE OR SILENT DISCHARGES

1. *The ozonizer discharge or the Siemens ozonizer*

As can be seen from A in figure 1, two electrodes, both separated from the reaction space by a glass wall, constitute an ozonizer. Ordinarily

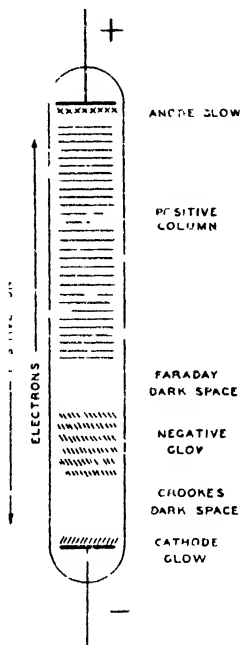


FIG. 2 Details of the glow discharge

it is constructed from tubes, although other forms have been used. It is also possible that dielectrics other than glass could be employed to separate the electrodes from the reaction space.

Ordinarily, voltages from about 5 to 25 kilovolts are used to operate the ozonizer. Various frequencies have been used; at zero frequency the discharge does not occur, but quite satisfactory discharges occur from about 50 cycles per second on up.

The discharge can be operated at high or low pressures and with liquids or gases.

2 and 3. The corona discharges

"When the electric field around a point or wire becomes sufficiently high for break-down at these regions before a spark can propagate across the gas space a corona discharge around the point or wire occurs." (172, page 485.) The corona discharges can occur at any frequency, including zero, and at voltages that are determined by the physical character of the apparatus and the conditions of pressure and temperature.

4. The glow discharge

When sufficient voltage is applied to two electrodes in a reaction space at a pressure of from 0.01 to 100 mm. (usually about 5 mm.), a glow discharge will occur. When conducted at zero frequency the discharge assumes the form given in figure 2. The voltage required varies from about 100 volts upwards and depends on the pressure and composition of the gas in the discharge.

The physical aspects of this discharge have been studied very extensively, so that most of the characteristics of the discharge are known. These characteristics, so pertinent to the electrical reactions of hydrocarbons, are considered in detail in the section on theory.

5. The electrodeless discharge

A conducting coil (inductance) through which a high-frequency current is flowing can cause a discharge to take place in an adjacent gas if the gas is of the correct pressure and the intensity of the electromagnetic field is great enough. Ordinarily, the inductance is wound around the outside of a glass discharge vessel. The pressure conditions inside the flask must be within the range of 0.01 mm. to 100 mm. for the discharge to occur. In most respects this discharge resembles the glow discharge, except that it will operate only at high frequencies.

III. REACTIONS OF PURE HYDROCARBONS IN THE NON-DISRUPTIVE OR SILENT DISCHARGES

A. OLEFINS

Of the various types of hydrocarbons, the olefins are among the most reactive chemically. This is also true in the silent discharges. Since these reactions can be made to occur under comparatively mild conditions, the reactions of the olefins offer the best opportunity for obtaining an insight into these electrical reactions. Once the reactions of the olefins are understood, the reactions of other hydrocarbon types can better be approached.

Most of the study on the reactions in the electric discharges has been

qualitative in nature rather than quantitative. The reason for this is that the variables involved are not easily measured or controlled. Probably the most important variables are (1) effective discharge intensity and (2) effective residence time in the discharge. The effective discharge intensity may be likened to temperature in thermal work and the residence time to contact time. The discharge intensity is a function of the voltage, frequency, current, and structural characteristics of the apparatus, as well as the pressure in the discharge space.

The limitations of analytical methods for determining the nature of the product also restrict the results to qualitative rather than quantitative interpretation. The gases can be analyzed with considerable accuracy, but the methods for the liquid products are inadequate. It seems quite likely that many, if not most, of the liquid products are complex mixtures that would have to be analyzed by proximate methods to give the type of hydrocarbons present, e.g., acetylenes, olefins, aromatics, naphthenes, and paraffins. Even this has not been done in most cases.

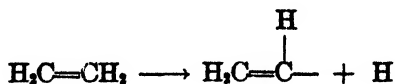
1. Ethylene

Considerable qualitative and exploratory work on ethylene has been done. The products most frequently identified have been hydrogen, acetylene, and a liquid. This liquid varies in nature from a thin, mobile, colorless product, which is probably a mixture of simple polymers, to high-boiling oils and solids. A summary of these studies is given in table 1, where the type of discharge and the reaction products are given.

The results given in table 1 indicate the complexity of the reactions taking place in the silent discharges. It is obvious that (1) the primary reaction products are subjected to secondary reactions; (2) more than one primary reaction occurs; or (3) both 1 and 2 may take place. It seems worth while to try to establish which occurs.

In the glow discharge radicals are formed that are capable of removing antimony or lead mirrors, but not zinc or cadmium mirrors. The radicals from ethylene have not been identified. This is quite an interesting observation, for ethylene does not give radicals that remove such mirrors when treated thermally under conditions that give such radicals from saturated hydrocarbons (213).

The present writers suggest that these radicals from ethylene in the discharge are vinyl radicals and that they are produced by breaking a carbon-hydrogen bond:

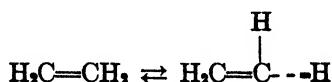


A rapid formation of acetylene in the ozonizer discharge has been observed as an initial reaction (241). The vinyl radical could give this acetylene. On the other hand, when high flow rates were used and the products immediately cooled to -60°C ., 1-butene and 1-hexene were found in 90 to 95 per cent yields (188). These compounds are simple polymers of ethylene. They could be built up by adding the vinyl radical to other ethylene molecules with later saturation. Alternatively, it seems quite possible that the carbon-hydrogen bond that is broken to give

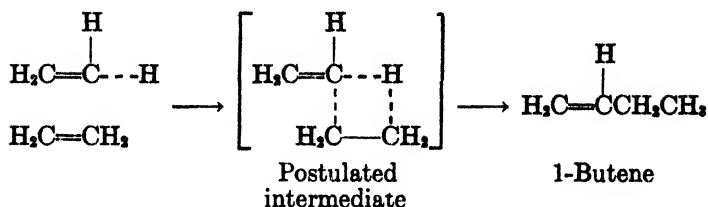
TABLE 1
Ethylene in the ozonizer-type discharge

REACTION PRODUCTS	REFERENCES
Colorless liquid product	(251)
H_2 , C_2H_2 , and a liquid product	(34, 38)
H_2 , C_2H_2 , C_2H_4 , liquid $(\text{C}_8\text{H}_{14})_n$, and resins	(42)
At -20°C ., H_2 , liquid mixture (b.p. $100-250^{\circ}\text{C}$), and a rubber-like solid analyzing close to $(\text{C}_8\text{H}_8)_n$	(54)
Yellow oil, b.p. $> 200^{\circ}\text{C}$	(181)
Yellow oil $(\text{C}_{12}\text{H}_{22})$, b.p. $> 260^{\circ}\text{C}$.	(174)
Liquid, $\text{C}_{14}\text{H}_{26}$, b.p. $100-110^{\circ}\text{C}$. at 14 mm.	(179)
Solid $(\text{C}_{16}\text{H}_{30})_n$, m.p. 105°C	(179)
Solid $(\text{C}_{16}\text{H}_{30})_n$, m.p. 110°C .	(179)
Liquid, $\text{C}_{20}\text{H}_{40}$	(133)
Liquid containing oxygen (probably absorbed oxygen from the air)	(135)
At -60°C , 1-butene and 1-hexene	(188)
Liquids: $\text{C}_{16}\text{H}_{30}$ and $\text{C}_{20}\text{H}_{40}$ before exposure to air; $\text{C}_{16}\text{H}_{30}\text{O}$ and $\text{C}_{20}\text{H}_{40}\text{O}_2$ after exposure to air	(177)
H_2 , C_2H_2 , C_2H_4 , 1- C_4H_8 , C_4H_{10} , liquid, and a resin	(222)
Polymers $(\text{C}_2\text{H}_4)_n$ to $(\text{C}_2\text{H}_4)_6$; a small amount of paraffins found in product, b.p. $90-210^{\circ}\text{C}$	(63)
H_2 , CH_4 , C_2H_2 , C_2H_4 , C_2H_6 , C_4H_{10} , and an unsaturated liquid, $\text{C}_n\text{H}_{1.5n}$	(164)
H_2 , C_2H_2 , C_4H_6 , C_4H_8 , and C_4H_{10} plus C_6 -paraffins and C_6 -olefins	(247)
H_2 , C_2H_2 , saturated and unsaturated condensation products	(241, 263)
C_2H_2	(204)
Polymers	(269)

vinyl radicals is broken only in extreme cases and is activated in other cases. This can be represented as follows:



where C--H indicates an activated carbon-hydrogen bond. This molecule containing an activated bond can then react with another ethylene molecule:



From this discussion it seems certain that there are two primary reactions of ethylene in the silent discharge: (1) dehydrogenation to acetylene, and (2) polymerization.

The primary reactions having been established, it is worth while to examine the reaction products in more detail. The liquid product in some cases is known (133, 134) to absorb oxygen from the air quite readily. Ethylene polymers (i.e., monoölefins) do not absorb oxygen so readily, while the acetylene polymers are quite noted for this property. This suggests that one of the secondary reactions is the polymerization of acetylene.

Still another series of experiments throw light on the secondary reactions of ethylene. These experiments were made in an ozonizer using a high-frequency power source and the products were condensed by cooling to -70°C . The following products were obtained:

PRODUCT	PER CENT
Uncondensed (H_2 , C_2H_2 , C_2H_4)	13
Butane	45
1-Butene	15
Fraction boiling $35-45^\circ\text{C}$.	4
C_6 -fraction	15
Higher hydrocarbons	8

The C_6 -fraction contained 1-hexene and paraffin hydrocarbons, presumably hexanes (222).

The saturated hydrocarbons are the interesting ones in this experiment. Are they formed by simple hydrogenation of the corresponding olefin? This is entirely possible. On the other hand, it is conceivable that the butane is formed by the alkylation of ethane with ethylene. There is nothing that will permit a selection, except that the low concentration (<13 per cent) of ethane might tend to favor the postulate of direct hydrogenation.

By using high-frequency current to energize the ozonizer, it was found that the nature of the reaction products could be controlled by the experimental conditions. In one case the major product was butene, in

another acetylene, and in still another butadiene. The energy consumption was 20 kw.-hr. per kilogram of ethylene reacted (247).

Low-boiling olefinic polymers were obtained by other workers (140) when an ozonizer excited by high-frequency current was used to treat ethylene. The exit gas analyzed as follows: 91 per cent H_2 and paraffins; 6 per cent C_2H_4 ; 3 per cent C_3H_6 and C_4H_8 . One gram-mole of ethylene reacted for each 2.3 kw.-hr. consumed.

The kinetics of the reaction of ethylene in the ozonizer discharge have been studied (163, 164, 165). There seems a little doubt that there is a definite induction period. The reaction resembles the reaction caused by alpha-particles (158).

In the high-frequency corona discharge, static ethylene reacted completely in 10 hr. The gaseous product contained 67 per cent of hydrogen and 20 per cent of saturated hydrocarbons. The liquid fraction was a dark oil with a molecular weight of about 500. An induction period of about 2 hr. was found, and it was noted that admixing hydrogen in the charge shortened the induction period and increased the reaction velocity. In a dynamic system, butadiene was found in the products condensed at $-85^\circ C$. The yield of butadiene was a maximum of 30 per cent of the ethylene reacted when the initial gas contained 23 per cent of hydrogen. Since the reaction had an induction period and was sensitive to an added gas (hydrogen), it was concluded that reaction occurred through a chain mechanism (13; cf. also 69).

In the semi-corona discharge, ethylene gave liquid products with densities from 0.78 to 0.82, molecular weights from 130 to 160, and n_D^{20} from 1.45 to 1.46. The yield was 0.204 g. of liquid per kilowatt-hour (161).

The electrodeless discharge in ethylene also gives hydrogen and condensation products (117).

2. Higher olefins

In the ozonizer propene is known to polymerize to a liquid product and the gas contains hydrogen and methane (30, 208). In one case the liquid had the following properties: $d = 0.824$, refractive index = 1.4578; average molecular weight = 233.

Complete analysis of the gases produced during the polymerization of propene in the ozonizer tells very little about the actual reactions taking place, for it can not be told whether the gas comes directly from the propene or from secondary reactions of the propene polymer. Since 85 to 90 per cent of the reacting propene is polymerized, the other reactions are of minor importance. Table 2 gives the moles of product per 100 moles of propene reacted (166).

2-Butene in the ozonizer gave an oil having an average molecular weight

of 202, a density of 0.831, and an iodine number of 156 (208). No gaseous products were reported.

In the high-frequency ozonizer propene polymerized, 2.3 kw.-hr. causing 1 gram-mole of propene to react. The liquid formed was mostly dimers and trimers. The exit gas analyzed as follows: 12 per cent C_2H_4 ; 50 per cent C_3H_6 and C_4H_8 ; 38 per cent H_2 and paraffins (140).

Isobutene polymerizes in the ozonizer to give a mobile yellow-brown liquid (63). A detailed investigation (208) revealed that the liquid had the following average properties: $d_4^{20} = 0.831$, $n_D^{20} = 1.4483$; molecular weight, 202; iodine number (Wijs), 156; C = 84.46 per cent; H = 14.60 per cent. Upon fractionation it was found that the largest fractions correspond to di- and tri-isobutene, although some of the evidence indicated that 2,3-dimethylbutane and 2,3-dimethyl-2-butene might be in

TABLE 2
Gaseous products from propene in the ozonizer discharge

Time, minutes.	24.5	73.5	73.5
Products, moles per 100 moles reacted:			
H_2	10.7	16.9	16.4
CH_4	3.7	6.1	6.8
C_2H_2	18.3	2.5	1.7
C_2H_4 }		1.4	1.4
C_2H_6 }	11.1	1.8	2.5
C_3H_4	13.0	5.7	7.1
C_3H_6 }			
C_3H_8 }	13.9		
C_4H_{10} }			
C_4H_{12}	2.2	1.8	0.8
Per cent C_2H_4 reacted	48.2	94.2	95.1
Per cent of original C_2H_4 to liquid	20.4	74.6	77.3

the 32–52°C. fraction, and that 2,2-dimethylpentane, 2,4-dimethylpentane, 2,2,3-trimethylbutane, and the related olefins might be in the 75–85°C. fraction. The higher fractions are richer in olefins than the lower boiling fractions; these higher fractions also contain naphthenes, but apparently no aromatics.

To workers studying fuels for internal-combustion engines, it would be interesting to have data on the antiknock properties of the products produced by the electrical polymerization of isobutene. Similar data on the completely hydrogenated product would be interesting, as it is a potential aviation fuel. From the data given above, it seems that isobutene polymerizes as easily electrically as it does catalytically, so that it should not be too difficult to obtain sufficient product for these tests and, if the product has useful properties that are not easily obtainable catalytically, it should not be difficult to produce such products on a large scale.

"Amylene" from fermentation amyl alcohol has been studied both in the ozonizer discharge and in the semi-corona discharge. A summary of the results is given in table 3 (185).

In the presence of hydrogen the three isomeric pentanes, various pentenes, isopropylacetylene, and other products were observed. (So far as the writers know, this is the only case in which neopentane has been reported as a product of electrical action on a hydrocarbon. In this case

TABLE 3
Products obtained by the electrical treatment of "amylene"

TYPE OF DISCHARGE	CARRIER GAS	AMYLENE CHARGED IN PRODUCT		PRODUCTS IN GAS			
		Liquid	Gaseous	Saturated hydrocarbons	Olefins	Acetylene	Hydrogen
		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Ozonizer	H ₂	79.1	2.5	63.5	9.5	27.0	
Semi-corona.	H ₂	14.8	15.6	46.7	20.0	33.3	
Ozonizer	None	57.4	7.1	65.0	4.1	24.4	6.5
Ozonizer	H ₂	81.7	5.0	38.2	8.8	23.6	29.4

TABLE 4
Olefins in the glow discharge

OLEFINS	ANALYSIS OF GAS IN VOLUME PER CENT				dp/dt	W
	Hydrogen	Acetylenes	Olefins	Paraffins		
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>		
1-Heptene	36.3	24.1	25.4	12.3	115	0.77
2,2,3-Trimethylbutene	37.9	7.9	26.9	27.3	97	0.58
(?)-Octene	38.7	14.0	36.4	10.9	105	0.80
Diisobutene	57.3	11.0	13.8	17.9	139	1.19

dp/dt = rate of gas formation, in cubic centimeters per milliamper second $\times 10^4$.

W = rate of solid formation, in grams per milliamper second $\times 10^4$.

the evidence is the boiling point of a few drops of material, without other supporting data.) The acetylene in the gas included not only acetylene, but also propyne, vinylacetylene, and diacetylene. Thus the reactions taking place probably include polymerization, isomerization, cracking, dehydrogenation, and, especially in the presence of hydrogen, hydrogenation (185).

Two main differences were noted between the reactions in the ozonizer discharge and in the semi-corona discharge: (1) For a given energy input

the amount of reaction taking place in the semi-corona was much more than the amount in the ozonizer, and (2) the changes in the semi-corona were more deep seated with more gas formation and carbon deposition on the wire electrode (185).

A few of the liquid olefins have been studied in the glow discharge, the low pressure in this type of discharge permitting the study of the vapor at room temperature. In this study only the gaseous products were analyzed. As can be seen from table 4, dehydrogenation is the chief reaction, the reduced pressure in the reaction zone, the high molecular weight of the olefin, or both operating to reduce the polymerization and condensation reactions observed with the lower olefins (170).

3. Butadiene

In the glow discharge at 10^{-3} to 10^{-1} mm. butadiene decomposes without evidence of polymerization. Above 10^{-1} mm. polymerization predominates. The constitution of the polymer is not known. When butadiene is reacted in the presence of hydrogen, polymerization also occurs. An induction period of 3 to 7 min. and the acceleration of the reaction rate by oxygen and argon and by increasing the size of the vessel were considered sufficient evidence for a chain mechanism (221).

The treatment of butadiene in the high-frequency ozonizer gave a liquid product that was thought to contain cycloolefins. The exit gas from the ozonizer analyzed as follows: 46.67 per cent ethylene; 25.4 per cent C_4H_6 and C_4H_8 ; 28 per cent hydrogen and paraffin hydrocarbons (140).

B. ACETYLENES

As might be expected from the work on olefins just discussed, acetylene has a strong tendency to polymerize when subjected to the silent discharges. Table 5 gives a summary of the available work on acetylene. The liquid and solid products obtained from acetylene by electrical treatment have a powerful tendency to absorb oxygen from the air (134, 135, 180). This makes the study of these products rather tedious, if it is desired to study the unchanged products. Considerable confusion has resulted in the past, because oxygen would be absorbed unsuspected by the worker, and when carbon and hydrogen were determined by combustion the total would not equal 100 per cent. A special analytical technique has been evolved to cope with this situation (97).

When acetylene reacted in a high-frequency ozonizer discharge and the reaction products were cooled to $-60^{\circ}C$. before recycling, a 70 per cent yield of a colorless liquid was obtained (187, 188, 222). This liquid had the molecular weight of the trimer and from the reactions of the liquid it

was concluded that it contained 1,5-hexadiyne, methylpentadiyne, and 1,5,3-hexadienyne. The liquid had a boiling point of -10°C . at 23 mm., $n_D^{20} = 1.4446$, and $d_4^{20} = 0.752$; it polymerized further on standing at room temperature, oxidized at room temperature when in contact with the air, and exploded on heating. These products are all isomeric with benzene, but no benzene was found. Both the density and the refractive index of the product argue against the presence of appreciable amounts of benzene.

TABLE 5
Acetylene in the ozonizer-type discharge

REACTION PRODUCTS	REFERENCES
Yellow oily liquid	(269)
Liquid and solid	(252)
Thick brown liquid, a brown solid, and a little gas consisting of 92% H_2 , 4% C_2H_4 , and 4% C_2H_2	(29, 31, 32)
Resinous solid containing oxygen	(229)
Brown "semi-solid" that absorbed oxygen	(127)
Thick brown liquid that later solidified	(174)
Insoluble solid	(133)
Hydrogen, carbon, a liquid that explodes when heated above 100°C , and a yellow-brown solid	(179)
"Warm" apparatus: a liquid and a solid	(137)
"Cold" apparatus: only a liquid, $(\text{C}_2\text{H}_2)_n$	(137)
In the presence of hydrogen (optimum $1\text{C}_2\text{H}_2:4\text{H}_2$), acetylenes to C_{10} and paraffins to C_8	(266)
Brown condensation product in the presence of hydrogen; hydrogen absorbed	(164, 165)
At -60°C ., 70% to trimers: 1,5-hexadiyne, methylpentadiyne, and 1,5,3-hexadienyne	(187, 188, 222)
Solid that absorbs oxygen	(138)
Condensation products	(83)

It has been found that only liquid products are obtained if the reaction vessel is kept "cold," while both liquid and solid products are found if the vessel is kept "warm." The reactions of both liquid and solid indicated the presence of benzene derivatives in which unsaturated side chains were probably attached to the benzene ring (137). This type of product could be formed by the cyclization of the higher acetylene polymers.

While the products from acetylene are absorbing oxygen from the air, it has been found that a photographic plate becomes exposed and the iodine is liberated from a solution of potassium iodide (137, 175). Both of these actions stop as soon as the absorption of oxygen ceases.

From this work it seems that the primary action of the silent discharge on acetylene is to form polymers. If the reaction products are quickly removed from the system, relatively simple polymers are formed. Further reaction may result in the formation of long-chain, highly unsaturated aliphatic compounds, or cyclization may take place with the formation of aromatic hydrocarbons with unsaturated side chains.

In the presence of hydrogen, acetylene reacts faster than acetylene alone. After the reaction has proceeded for a while, the effect decreases and the reaction finally proceeds at the normal rate. In the presence of inert gases, the rate of reaction is proportional to the acetylene concentration. The reaction products were not given (242).

In another study, in the presence of hydrogen, a complex mixture of liquid products was obtained. This was said to contain acetylenic hydrocarbons up to C_{10} and saturated hydrocarbons to C_8 . At reduced pressures less polymerization and more hydrogenation took place (266).

In the presence of benzene, acetylene reacted only slightly differently from acetylene alone. There is no evidence that benzene took part in the reaction (176). It has been claimed that methane and acetylene interact in the silent electric discharge to give as high as 70 per cent yields of propene (109).

It has been found that increasing the wave length from 20 to 48 meters increases the amount and rate of the reaction of acetylene (65).

When compared in ozonizer apparatus of the same type and under identical conditions, it was found that the relative rates of reaction were as follows: acetylene, 20; ethylene, 10; methane, 2 (165).

In the electrodeless discharge acetylene gives a solid product, which has been variously reported as a yellow-white powder (191), an insoluble red-brown solid (105, 106), and polymers (and hydrogen) (117); no mention is made of the solid in one case (11).

One of the noticeable features of various electrical discharges in gases is that light is emitted. Quite often very beautiful color effects are present. A spectrometric examination of the light produced when acetylene is in the discharge indicates the presence of C^+ ions, carbon atoms, and hydrogen atoms (105, 106, 141). We do not know the chemical reactions of such fragments as C^+ ions and carbon atoms; hence the difficulty of proposing a complete reaction mechanism which is related to the actual processes occurring.

From the foregoing discussion, one may judge that acetylene is quite reactive in the electrical discharges. This is of special significance if acetylene is to be made by treating petroleum hydrocarbons in the electric discharge, for it means that the acetylene should be removed from the

reaction zone as soon as formed, otherwise the yield will be reduced by secondary reactions. These secondary reactions may be minimized by using the electric discharge at subatmospheric pressure and limiting the amount of reaction during the passage through the discharge.

Higher acetylenes

So far only results on the ozonizer treatment of 1-heptyne and 1-octyne have been reported. 1-Heptyne is said to give "diheptyne" (a colorless mobile liquid), "triheptyne" (a thick odorous oil), and "undecaheptyne" (a dark red mass insoluble in ether or benzene) (179).

1-Octyne produced "dioctyne" (a colorless liquid) and "nonaoctyne" (a dark red soft mass soluble in ether and benzene but insoluble in alcohol) (179).

The heavy viscous polymers obtained are apparently complex mixtures having an average molecular weight corresponding to undecaheptyne and nonaoctyne, respectively.

No gaseous products have been reported as the result of the action of the silent electric discharge on the higher acetylenes.

C. AROMATIC HYDROCARBONS

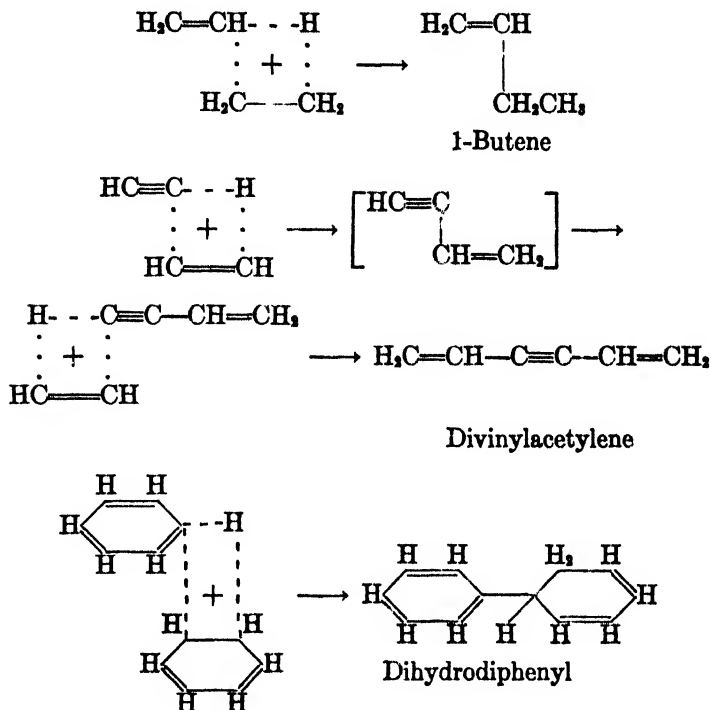
1. Benzene

Diphenyl is the most common identifiable reaction product reported to result from the action of electrical discharges on benzene (12, 62, 117). More complex products of a resinous nature are also formed, which have a composition closely approaching $(CH)_x$. Such products readily absorb oxygen from the air, and in some cases apparently combination with water vapor seems to occur. Such action brings to mind the reactions of the products from acetylene. Since acetylene is formed in the discharge and may itself react, the finding of such products is to be expected (12, 105, 106, 169, 176).

Hydrogen and acetylene are the most common gaseous reaction products. Table 6 gives a summary of the reactions of benzene.

When benzene is treated in the ozonizer under mild conditions, diphenyl is produced, along with a liquid fraction boiling in the same range as diphenyl. This liquid can be separated from the diphenyl by cooling to about $-30^{\circ}C.$, when the diphenyl crystallizes. Upon analysis it was found that the liquid contains more hydrogen than corresponds to diphenyl. From the data available, it was concluded that this liquid product was probably a mixture of dihydrodiphenyls. Although this product might appear a little unusual at first, it can be formed by reactions which

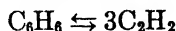
are analogous to those already observed in the case of ethylene and acetylene.



In view of this analogy, the reaction seems reasonable enough. It is to be regretted that the original workers (222) did not obtain additional evidence for the dihydrodiphenyl. For example, the dihydro ring contains a conjugated diene structure that should react readily with maleic anhydride to give a solid derivative. The dihydrodiphenyl was postulated as the intermediate between benzene and diphenyl in the discharge. From this, it seems that the action of the discharge was one of polymerization followed by dehydrogenation. A continuation of this process would account for the resinous substances formed in the discharge.

When the intensity of the discharge was increased, it was not possible to isolate the dihydrodiphenyl but only the diphenyl, indicating that the dehydrogenation to diphenyl with the formation of an aromatic ring takes place readily (222). It seems quite possible that this same mechanism for the formation of diphenyl could also apply to the thermal reactions of benzene in some cases.

Acetylene was also formed in the discharge, indicating that a depolymerization of benzene was also taking place.



From the ratio of the products the ratio of the two reactions has been calculated.

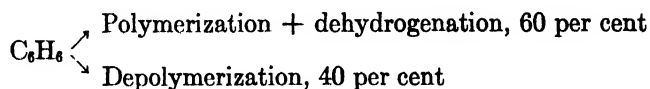


TABLE 6

Benzene in the ozonizer-type discharge

REACTION PRODUCTS	REFERENCES
Gummy condensation product	(181)
Gummy, wax-like material	(110, 111)
H ₂ , C ₂ H ₂ , and hydrocarbon gases	(110, 111)
An oil and a solid, both C ₂₄ H ₂₆ and both absorbing oxygen from the air	(176)
An oil, (C ₆ H ₆) _n , and a solid, C ₂₄ H ₂₆	(177)
Diphenyl and a solid condensation product	(12)
Diphenyl, <i>p</i> -diphenylbenzene, a resin (C ₆ H ₄) ₂ , and a gas consisting of 52.6% H ₂ , 29% C ₂ H ₂ , 12.1% C ₂ H ₄ , and 6.3% paraffins	(62)
Diphenyl (solid), dihydrodiphenyl (liquid), a solid brown resin, and a gas consisting of 52% H ₂ , 32.8% C ₂ H ₂ , 7.2% C ₂ H ₄ , and 0.8% higher olefins	(222)
Hydrogen plus benzene gives a product which absorbs oxygen. By steam distillation the product can be separated into an oil, C ₁₂ H ₁₄ , and a solid, C ₂₈ H ₃₄	(176, 177)
Methane plus benzene gives a yellow-red oil, C ₂₈ H ₃₆	(176)
Ethylene plus benzene gives a brown-red liquid, C ₂₈ H ₃₄	(176)
Acetylene plus benzene gives a yellow-brown solid, C ₄₈ H ₄₆	(176)

A spectrometric study of the light emitted by benzene in the electrodeless discharge indicates that C⁺ ions, carbon and hydrogen atoms, and C₂ and CH molecules are present (105, 106, 117). The reaction product is a brown, insoluble product, (CH)₂. Just how much of the material goes through these stages and just how much effect these active particles have in determining the reaction products is not known. From the fluorescence spectra it has been calculated that a considerable portion of the benzene molecules having vibrational energy greater than 5×10^{-8} ergs decompose (1). In the glow discharge, CH, C₂, C, H, and H₂ (no C⁺) were found by a spectroscopic study (107).

2. Benzene with other substances in the ozonizer

Methane. A yellow-red oil, the nature of which is unknown, was produced (176). Both methane and benzene reacted.

Acetylene. A solid and a liquid product was formed. So far as could be told, the solid was identical with that produced from acetylene alone. The liquid was different, and apparently also different from that obtained from benzene alone (176).

Ethylene. A brown-red liquid soluble in benzene and ether was produced. Apparently both ethylene and benzene react, although the ratio was not determined (176).

Hydrogen. This product was different from that produced by benzene alone, but the presence of the hydrogen did not destroy the ability of the product to absorb oxygen from the air (176). The product could be separated by steam distillation into a liquid and a solid. The liquid was colorless (b.p. 241–243°C.), while the solid was a clear red mass (177).

3. Toluene

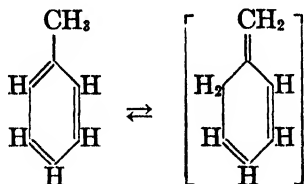
The reactions of toluene in the discharge resemble those of benzene qualitatively, with the exception that dibenzyl has been identified as one of the products.

In the ozonizer the products were a thick yellow liquid, boiling at 140–150°C. at 14 mm. and having the composition of a toluene dimer, together with a resin melting at 150°C (179). In the semi-corona discharge toluene gave a shellac-like deposit having a density of 0.95, a carbon content of 79.16 per cent, and a hydrogen content of 5.9 per cent. The rest of the composition was assumed to be oxygen. In other experiments dibenzyl, 2,2'-dimethyldiphenyl (m.p. 17°C.), and a liquid which boiled >200°C. and did not freeze at 0°C. were found. This last product was thought to be a mixture of isomers of dimethyldiphenyl (12).

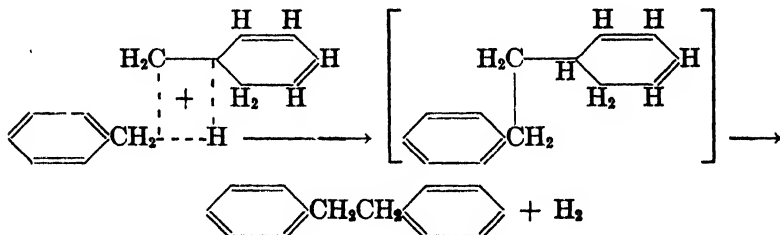
Under similar conditions in the ozonizer the composition of the gas from toluene was determined; this was hydrogen, 58 per cent; acetylene and homologs, 10 per cent; ethylene, 8 per cent; higher olefins, 3 per cent; ethane, 4 per cent; and methane, 14 per cent. Under identical conditions the rate of gas evolution was twice that observed with benzene. Besides the gas, a liquid boiling at 110–145°C. at 18 mm. and a brown resinous powder were formed. The liquid was separated into dibenzyl plus a residual liquid, boiling at 130–136°C. at 17 mm. and having the composition of a polymer of toluene (222).

Toluene can be depolymerized to acetylenes and also polymerized. The polymerized product may be dehydrogenated in the discharge to

give dimethyldiphenyls. In these respects toluene resembles benzene in its reactions. In addition to the reaction products analogous to those from benzene, dibenzyl has been found. By making a simple assumption, it is possible to write an equation explaining the formation of dibenzyl by means of reactions entirely analogous to those already presented. The assumption is that toluene is capable of the *allylic rearrangement* under the influence of the discharge. Structurally this change is represented as follows:



The addition of an activated toluene molecule to the methylene double bond, followed by dehydrogenation, gives dibenzyl.



The allylic rearrangement is thought to be ionic in nature, so that there seems to be no objection to its occurrence in an electric discharge. More evidence is needed before the assumption can have validity. For the time being, the mechanism can be justified only by known analogies and because it explains the product.

4. Higher aromatic hydrocarbons

A whole series of substituted benzenes have been studied in a glow discharge at reduced pressure. The rate of gas evolution and the composition of the gas were determined (170). A summary of these results is given in table 7. The original workers made the following observations in regard to the results: (1) For any given series the amount of gas produced increases with increasing molecular size. (2) Increasing centralization of the molecule results in decreasing amounts of gas produced. Example: the butyl-substituted benzenes. (3) Increasing proximity of substituted

groups in the benzene ring decreases the amount of gas produced. Example: the *o*-, *m*-, and *p*-xylenes.

Although the picture is somewhat complicated by secondary reactions, the principal primary ones seem to be as follows: (1) Polymerization of the hydrocarbon followed by dehydrogenation. The products are dehydropolymers (resins) and hydrogen. (2) Depolymerization to give acetylenes. (3) Dealkylations to give olefins and a simpler aromatic hydrocarbon. (4) Hydrogenation of a portion of the acetylenes to pro-

TABLE 7
Benzene and benzene derivatives in the glow discharge

AROMATIC HYDROCARBONS	ANALYSIS OF GAS IN VOLUME PER CENT				dp/dt	W
	Hydrogen	Acetylenes	Olefins	Paraffins		
	per cent	per cent	per cent	per cent		
Benzene	46.0	40.5	4.4	9.2	25	
Toluene	54.8	29.0	3.0	13.7	52	4.39
<i>o</i> -Xylene	60.2	16.0	7.8	16.0	35	1.69
<i>m</i> -Xylene	52.5	25.1	6.8	15.8	47	
<i>p</i> -Xylene	73.0	11.6	6.3	9.1	63	1.37
Mesitylene	55.1	18.9	7.4	18.7	71	2.43
Hexamethylbenzene	54.7	11.4	13.1	20.9	79	2.76
Ethylbenzene	50.7	19.6	12.4	17.3	64	2.82
<i>m</i> -Diethylbenzene	49.8	14.1	16.9	19.2	71	2.94
<i>p</i> -Diethylbenzene	36.0	11.4	25.7	27.0	69	1.77
Hexaethylbenzene	37.8	20.6	17.8	23.8	83	1.29
<i>n</i> -Propylbenzene	43.0	12.8	27.0	17.2	71	2.90
Isopropylbenzene	51.8	14.4	12.1	21.6	58	1.53
<i>p</i> -Cymene	46.2	12.0	16.6	25.2	66	2.09
<i>n</i> -Butylbenzene	56.4	9.6	18.0	16.1	81	2.91
<i>sec</i> -Butylbenzene	50.6	16.8	16.6	16.2	80	2.13
<i>tert</i> -Butylbenzene	45.5	16.8	11.9	23.8	66	2.73
Styrene	45.7	18.8	30.7	4.7	45	4.32

dp/dt = rate of gas formation, in cubic centimeters per milliamper second $\times 10^4$.

W = rate of solid formation, in grams per milliamper second $\times 10^4$.

duce olefins, and hydrogenation of a portion of the olefins to give paraffins. Besides the above work, a few of the aromatic hydrocarbons have been studied under different conditions.

Xylenes. In the ozonizer discharge the xylenes give "dixylenes" as thick yellow liquids, together with more complex products. The "dixylene" from *o*- and *p*-xylene boils at 160–170°C. at 14 mm., while that from *m*-xylene boils at 160–165°C. at 14 mm. (179).

Similarly treated, *p*-xylene gave a shellac-like product which, when

analyzed, was found to contain oxygen; it had a density of 0.97. In addition, di-*p*-tolylethane (m.p. 81°C.) was isolated, along with a high-boiling liquid thought to be made up of isomers of di-*p*-tolylethane (12). The formation of ditolylethanes from xylenes is analogous to the formation of dibenzyl from toluene.

The light from the electrodeless discharge in xylene was investigated spectrometrically. Evidence was obtained for the presence of CH, C₂, C⁺, and H in the discharge (107).

Mesitylene. The products from the ozonizer treatment at reduced pressure are a thick yellowish liquid, boiling at 195–200°C. at 14 mm., and said to be "dimesitylene", and a resin which analyzed correctly for (C₉H₁₂)₁₂ as the average composition (179).

A spectrometric investigation of the light from mesitylene in the electrodeless discharge indicates the presence of CH, C₂, C⁺, and H in the discharge (107).

Cumene. In a discharge of the ozonizer type, cumene gave dicumyl as a thick yellowish liquid, b.p. 162–165°C. at 14 mm., and a resin, m.p. 95°C., said to be "hexacumyl" (179).

p-Cymene. Under the same conditions *p*-cymene gave "dicymene" as a thick yellow oil, boiling at 185–190°C. at 14 mm., and a resin said to be "pentacymene", m.p. 80°C. (179).

Styrene. The application of an alternating current field to polymerizing styrene has no effect on the rate of polymerization until a critical potential gradient is reached. At 80°C. this is 43.5 kilovolts per millimeter. The polymerization rate is increased suddenly at this potential gradient, but the rate decreases if the gradient is increased further. A short preliminary treatment of the styrene at the critical gradient increases the thermal rate of polymerization and reapplying the field decreases the rate (157).

Naphthalene. Under the conditions used for cumene, naphthalene gave a brown, glistening, insoluble, infusible polymerization product (179).

In the electrodeless discharge naphthalene gave the same spectra observed with benzene. The lines were not equally intense when the same time of exposure of the photographic plate was used (105, 106, 107). This may have been due to a lower concentration of naphthalene in the discharge with the same conversion to light-emitting fragments or may have been due to a greater stability of naphthalene.

Polynuclear hydrocarbons have been studied at low pressures in the glow discharge. Under these conditions dehydrogenation and depolymerization to acetylenes takes place. The olefins formed probably result from the hydrogenation of acetylenes. Polymerization or condensation

to give solid products also occurs. A summary of the results is given in table 8 (170).

It will be recalled that in the thermal cracking of petroleum oils there can be produced a cracked gas oil of high density which is very refractory to further cracking. This product is said to be aromatic in character. It seems quite likely that refractory gas oil in a suitable electric discharge could be made to give commercial yields of acetylene. This is merely a conjecture based on the experimental data given above on the aromatic hydrocarbons. Nevertheless, the possibility is one of interest to the petroleum industry.

TABLE 8
Polynuclear aromatic hydrocarbons in the glow discharge

HYDROCARBONS	ANALYSIS OF GAS IN VOLUME PER CENT				dp/dt	W
	Hydrogen	Acetylenes	Olefins	Paraffins		
	per cent	per cent	per cent	per cent		
Diphenyl	43.2	37.4	17.3	2.1	43	5.65
Naphthalene	42.8	32.2	23.3	1.7	39	7.51
Stilbene	46.8	44.8	6.8	1.6	57	7.24
Anthracene	44.9	36.2	18.2	0.7	35	9.70
Phenanthrene	45.8	21.9	28.0	4.3	37	8.82
Acenaphthene	60.8	25.4	13.3	1.4	50	6.39
Triphenylmethane	50.1	27.3	21.8	0.8	42	6.91
Retene	39.8	21.1	15.0	24.1	56	5.08

dp/dt = rate of gas formation, in cubic centimeters per milliamper second $\times 10^4$.

W = rate of solid formation, in grams per milliamper second $\times 10^6$.

D. CYCLOPARAFFINS AND CYCLOOLEFINS

These types of hydrocarbons have not been studied under sufficiently mild conditions to indicate simple products which could be regarded as primary products. The more drastic conditions give products which are not characteristic of the original hydrocarbon.

1. Cyclopropane

This hydrocarbon reacts one-half as fast in the ozonizer discharge as propene under comparable conditions (208). The main reaction is condensation to give a liquid product of the empirical formula $C_{15}H_{26}$. This product was thought to be identical or isomeric with the product obtained from propene. For each 100 volumes of cyclopropane charged, 37.3 volumes of hydrogen and 1.5 volumes of methane were formed (30).

2. Cyclohexane

Cyclohexane has been studied in the glow discharge (170) and the gaseous products partially analyzed (table 9). Some work has also been done in the electrodeless discharge (11, 12), but no definite product has been reported. The solid, brown, resin-like deposit usually formed in the electrodeless discharge in hydrocarbons was almost absent when cyclohexane was used.

TABLE 9
Cycloparaffins and cycloolefins in the glow discharge

CYCLOPARAFFINS AND CYCLOOLEFINS	GAS ANALYSIS, VOLUME PER CENT				dp/dt	W
	Hydro- gen	Acety- lenes	Olefins	Paraffins		
	per cent	per cent	per cent	per cent		
Cyclohexane	46.0	13.2	32.1	8.7	86	0.51
Methylcyclohexane	47.0	12.6	26.6	13.8	93	0.19
p-Menthane	52.5	1.4	28.1	18.0	71	0.90
Decalin	52.5	12.8	30.0	4.7	103	1.52
Cyclohexene	48.7	16.5	30.0	4.8	105	1.06
Isobutylcyclohexene	40.8	12.6	31.1	15.5	105	1.36
Dipentene	48.4	11.5	27.0	13.1	114	1.66
Pinene	53.7	16.2	18.2	12.0	101	1.88
Limonene	58.4	14.9	18.0	8.7	112	1.62
Dihydronaphthalene	54.8	23.4	19.7	2.1	72	5.40
Tetralin	57.0	17.0	20.0	6.0	71	

dp/dt = rate of gas formation, in cubic centimeters per milliamper second $\times 10^4$.

W = rate of solid formation, in grams per milliamper second $\times 10^4$.

3. Pinene

The ozonizer discharge produces a small amount of diterpene (polymer) but no gas. The polymerization, although slow, was especially clean cut in a carrier gas (hydrogen or nitrogen) (30). Another worker (179) obtained a dimer and a heptamer by treating pinene in the ozonizer.

4. Camphene

In the ozonizer, camphene gave a dimer and an octamer (179).

5. Limonene

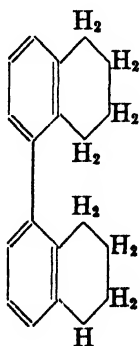
Limonene and the ozonizer gave a liquid dimer, a solid hexamer, and an insoluble solid (179).

6. *Menthene*

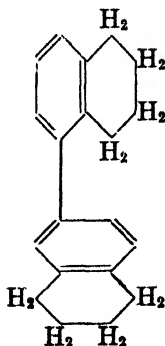
A liquid dimer and a soft brown polymer were obtained by treating menthene in the ozonizer (179).

7. *Tetralin*

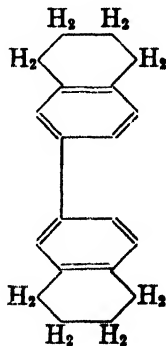
In the presence of air as an auxiliary gas, liquid tetralin gave hydrogen cyanide and probably other nitrogen-containing products when treated in the ozonizer discharge. When hydrogen was used, a part of the tetralin was converted into a thick, almost resinous product. This product was interesting because it possessed air drying properties similar to linseed and tung oils. Owing to the fact that the glass walls of the apparatus became pitted and soon suffered electrical breakdown, the apparatus was changed to the corona type with the inner conductors made of copper. Under these conditions the yield of resinous product was 22.1 g. per kilowatt-hour. Apparatus of this type was also used with tetralin vapor at 5 to 8 mm. pressure. The discharge consumed about 75 watts at 500 cycles. The apparatus was then further changed to the glow type with the electrodes made of copper. With operating conditions of 100 watts, 1 to 2 mm. pressure, 50 cycles, and 2000 volts the resin yield was 75 g. per kilowatt-hour. In a still larger apparatus, using 200 watts at 9550 volts, a small amount of a diteteryl ($C_{26}H_{22}$), m.p. 80–81°C., was isolated (19); just which one of the nine possible diteteryls is not known. If it should be proved to be one of the following compounds:



5,5'-Diteteryl



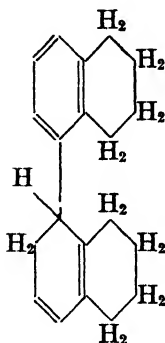
5,6'-Diteteryl



6,6'-Diteteryl

then it is entirely possible that this product is formed in a manner entirely analogous to the formation of diphenyl from benzene in the electric discharge. In this event it seems quite likely that the resinous product

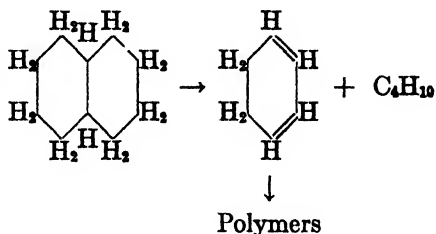
having air drying properties would be analogous to the dihydrodiphenyl formed from benzene.



This product would be a dihydroditetryl. Its ability to absorb oxygen from the air and polymerize is entirely consistent with such a structure. Until more work is done on the constitution of these products, these possibilities must remain speculative. Nevertheless, they may be useful in directing the work in trying to identify these products. (2,2'-Ditetryl, m.p. 113°C. (42), and 2,6'-ditetryl, m.p. 53-54°C. (228), are the only ditetryls of known structure.)

8. Decalin

In the ozonizer discharge, decalin gives a gas thought to be butane mixed with hydrogen and unsaturated hydrocarbons. In addition, 25 per cent of material boiling above decalin was formed. A fraction corresponding to 15 per cent of the decalin boiled above 200°C. at less than 1 mm. pressure (19). The reaction was thought to take place according to the scheme:



In another study by a different worker (92) it was found that the primary action of the discharge is the splitting off of hydrogen and the polymerization of the resulting unsaturated compounds. When the reaction is

carried out in the presence of oxygen, ozonides are formed; in the presence of nitrogen, nitrogen-containing products are formed.

Several of the cycloparaffins and cycloolefins have been studied in the glow discharge at reduced pressure. The powerful dehydrogenating action of the discharge is indicated by the data summarized in table 9. Cracking reactions also occur, since gaseous olefins, acetylenes, and paraffins are formed. More could be told about the reaction mechanism if the gas analysis were more complete, giving the individual components instead of the hydrocarbon groups (170).

It is well to note the gases produced in the glow discharge with tetralin and decalin (table 9). The gaseous reaction products are not those that would be expected if the reactions in the glow discharge were similar to those in the ozonizer discharge discussed above. Since the analyses are incomplete in both cases, definite conclusions may not be drawn, but it seems that the glow discharge is the more violent in nature, giving more deep-seated changes in the molecule. The present differences serve to emphasize our contention that in any discussion of the electrical reactions it is imperative that the type of discharge used be given as part of the essential data.

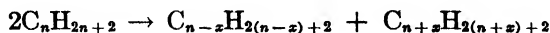
There are immense supplies of cycloparaffinic (naphthenic) petroleum oils which might make useful products. The above discussion indicates drying oils for paints as one possibility. Generally speaking, however, the reactions of these hydrocarbons in the electrical discharge need careful study, since the proper data are not available on the reactions of the most common members of this group of hydrocarbons, i.e., the cyclohexane and cyclopentane derivatives. Thus, as far as the chemist is concerned, the field is wide open, from the discovery of the fundamental reactions of these hydrocarbons all the way to the commercial utilization.

E. PARAFFIN HYDROCARBONS

In many respects the electrical reactions of the paraffin hydrocarbons resemble the corresponding thermal reactions. It will be recalled that, thermally, the paraffins react by dehydrogenation and by cracking and that this primary action is followed by the reactions of the olefins produced by the dehydrogenation or cracking. This same sequence of events seems to take place in the electrical reactions, with this difference: the electrical reactions of the olefins produced in the primary action are different from the thermal reactions. The reactions of the olefins have been purposely discussed before the paraffins in order to give a better understanding of the more complicated overall reactions of the paraffins. In this connection it must be kept in mind that the paraffins are less reactive electrically and therefore require a relatively more intense discharge to make them react

at a usable rate. This intense discharge often produces deep-seated changes in the olefins formed in the primary reaction, so that it is difficult to follow the reaction.

In addition to dehydrogenation and cracking, which can be obtained thermally, a type of reaction can occur electrically which does not occur thermally or which is poorly defined. Generally the reaction may be written:



So far as the present authors know, no name has been assigned to this reaction. It is a type of disproportionation.

1. Methane

Methane alone can react chemically only by dehydrogenation. In thermal reactions, methane is known to give the reaction

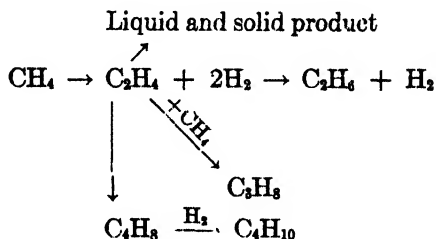


when caused to react by a heated filament located in a bulb cooled by liquid nitrogen. If the bulb is cooled with liquid oxygen ($-183^\circ\text{C}.$) instead of liquid nitrogen ($-195^\circ\text{C}.$), then acetylene and ethylene form up to 90 per cent of the hydrocarbon reaction product (240). The analogous experiment using the glow discharge with liquid air as the cooling medium indicates that the methane which reacts is quantitatively converted into hydrogen and ethylene (44). In view of the critical effect of the wall temperature in the thermal reaction, it can be argued that ethane and hydrogen might be the initial reaction products in the electrical reactions had the experiment been conducted in liquid nitrogen instead of liquid air. In the absence of data to the contrary, ethylene will be considered the primary reaction product from methane in the electric discharge. On this basis the electrical reaction products of methane would be those of ethylene in the presence of an excess of methane and hydrogen. As near as can be determined from the data available, this is the case. In discussing these data, it must be remembered that methane is one of the very stable hydrocarbons, and any discharge which is powerful enough to change it chemically produces profound changes in the reaction products as soon as these are present in appreciable concentrations.

From a study of methane in the ozonizer discharge at room temperature, the following observations were made (164, 165): (1) The volume of gaseous products equals the volume of the original methane. (2) An induction period of 20 to 25 min. exists. (3) The gaseous product is primarily hydrogen with relatively small amounts of C_2H_6 , C_3H_8 , and C_4H_{10} . Still smaller amounts of C_2H_4 have been found. From 20 to 55 per cent of

the reacting methane is converted into the above products; the rest is converted to solid and liquid products. (4) A liquid product is formed whose composition agrees closely with C_nH_{2n} . The liquid is apparently unsaturated, since it is acted on by light and air. (5) The liquid product left in the field of action of the discharge will react with evolution of gas.

The chemistry involved in these processes can be expressed in the following diagram.



In experiments at room temperature (222) in the ozonizer, there may be evidence that ethane is a primary product in the treatment of methane. Certainly ethane is formed in larger amounts than ethylene, although this can be readily explained, since ethylene is known to be much more susceptible to reaction than ethane (166).

Methane also gives liquid and solid products in the semi-corona discharge. Table 10 gives a summary of the conditions used and the results obtained. The apparatus consisted of eleven semi-corona tubes in series for gas flow and in parallel electrically. The central aluminum electrode was $\frac{1}{8}$ in. (0.32 cm.) in diameter and was enclosed in a Pyrex tube 2 cm. in diameter with 1 mm. wall thickness. Traps cooled in ice water were placed between the semi-corona units. The discharge was operated by an 18-kilovolt transformer (161).

Among the earliest products observed from the treatment of methane in the ozonizer discharge were acetylene and hydrogen (27, 25, 30, 222). It seems quite likely that acetylene is a secondary product resulting from the action of the discharge on the ethylene produced from methane.

Owing to the economic importance of acetylene, considerable study has been devoted to the conversion of methane to acetylene. Electrical methods have furnished their share of results in seeking the solution of this problem. The glow discharge has received most attention in this direction. It seems advantageous to heat the discharge tube to about 500°C. The best operating pressure is 40 to 50 mm. and the best electrode distance is 40 to 50 cm.; greater distances cause the formation of higher paraffin hydrocarbons. The voltage does not seem to be critical so long

as it is sufficiently high to maintain the discharge. The yields of acetylene are as high as 90 per cent of the methane charged. The energy consumption is 12 to 13 kw.-hr. per cubic meter of acetylene formed (85, 149, 151, 205, 206, 223).

When the above process was altered to produce maximum yields of ethylene instead of acetylene, the energy consumption at the point of maximum yield was 53 kw.-hr. per cubic meter of ethylene (206). The methane was diluted with 0.5 volume of hydrogen. Since the formation of ethylene from methane requires less energy from the thermodynamic point of view than the formation of acetylene, the reason for the larger energy consumption in the case of ethylene formation is not clear. It is possible that a considerable portion of the energy goes to form atomic hydrogen which recombines at the walls of the containing vessel, resulting

TABLE 10
Methane in the semi-corona discharge

Flow, liters per hour	0.57
Time, days	5.1
Methane used, liters	64.0
Methane used, grams	457
Liquid yield, grams	1825
Molecular weight of liquid	130-170
Density of liquid	0.78-0.83
Refractive index of liquid	1.44-1.46
Solid on glass walls, grams	4.4
Energy used, kilowatt-hours	102.7
Carbon on aluminum rods, grams	0.25
Grams of liquid per kilowatt-hour	0.178
Grams of liquid and solid per kilowatt-hour	0.22
Grams of liquid per gram of methane	0.40

in a temperature rise of these walls. In this case the energy would be dissipated as heat without doing useful chemical work.

The reaction kinetics for methane in the glow discharge have been treated mathematically (262), and the agreement with the published experimental results is said to be good.

A mixture of methane and an excess of nitrogen was studied with a thyatron circuit capable of giving a glow discharge lasting 10^{-5} sec. By varying the constants of the circuit, the number of these pulse discharges could be varied up to 133 per second. Acetylene, hydrogen cyanide, and a solid approximating $(CH)_x$ were formed. The following experimental observations were made under these conditions: (1) For the same watt input, the acetylene yield was greater with the pulse discharges than was obtained with A.C. or D.C. (2) With a D.C. or A.C. dis-

charge, practically all the carbon of the methane could be converted into hydrogen cyanide. Little or no polymers were formed. (3) With a pulse discharge rate of 10 per second an appreciable amount of brown, shellac-like, insoluble polymer was formed. (4) As the pulse rate was increased from 10 to 60 per second, the $\text{HCN}:\text{C}_2\text{H}_2$ ratio increased linearly with the pulse rate. (5) With pulse rates from 60 to 133 (the limit of the apparatus) per second, the $\text{HCN}:\text{C}_2\text{H}_2$ ratio was constant.

In explaining these observations, it was assumed that CH was the major active particle formed in this discharge. This CH particle may polymerize to C_2H_2 or $(\text{CH})_n$, or may react with nitrogen to form hydrogen cyanide by further reaction in the discharge. Since 60 pulses per second were sufficient to utilize all the CH particles, it was suggested that the life period of the particle was of the order of $\frac{1}{60}$ second (270). While this observation on the apparent life of the CH particle is interesting, it is preferred to wait for a more specific description of the experimental details before definite conclusions are drawn. More definite evidence was offered for the methyl radical, for dimethylzinc was formed when the gas was withdrawn through a hollow zinc cathode.

Indirectly, the effect of frequency has been studied in the more conventional glow discharge, using a variable electrical condenser. In the pressure range 1 to 11 mm. of mercury, the amount of methane reacted was a maximum at the maximum condenser capacity, (i.e., at the lowest frequency). At 3.2 mm., 75 per cent of the reacting methane was converted into acetylene, utilizing about 15 per cent of the available electrical energy (192).

Similar experiments at atmospheric pressure using frequencies of 6 and 15 megacycles gave results which indicated that the lower frequency gave a greater per cent conversion, while the higher frequency produced more deep-seated changes in the methane that reacted (204).

Besides the effect of the type of electrical discharge, it has been found that the electrode material may have influence. For example, in the glow discharge under a given set of conditions, it has been found that aluminum, copper, zinc, lead, and iron electrodes gave 1.5 per cent conversion of methane. Mercury electrodes gave 5.2 per cent conversion. Warming the mercury electrodes increased the conversion to 14 per cent. It was concluded that the mercury acted catalytically, probably in the vapor phase (147).

In the spectroscopic investigation of the light produced by the glow discharge in methane, evidence was obtained for H, CH, and H_2 . In the electrodeless discharge, evidence was obtained for C and C_2 , as well as H and CH. Brown to black resins were produced on the walls of the discharge vessel in both types of discharge (104, 105, 106, 107).

2. Methane with other hydrocarbons

Acetylene. The silent discharge gave a clear yellow liquid product which could be separated into a viscous liquid soluble in ether and an insoluble solid. The liquid had an average composition corresponding to C_6H_{10} , while the solid corresponded to C_6H_8 (174). Propene has also been reported (108, 109).

Ethylene. Under the same conditions, a 1:1 mixture of methane and ethylene gave a viscous liquid which absorbed oxygen from the air (174).

Benzene. A product was obtained which had the average composition $C_{28}H_{36}$ (176).

3. Ethane

Ethane can undergo both dehydrogenation due to activation of the C—H bond and demethanation due to activation of the C—C bond. The ratio of these two reactions has not been accurately determined. At the beginning of the reaction, in the ozonizer discharge, there is about five times as much dehydrogenation as demethanation, as determined from the gaseous products (222). After the discharge has acted on a given sample of gas for longer times, the amount of methane increases in the gas (159, 222). It seems entirely possible that the primary reaction of ethane would be dehydrogenation if experiments were made under conditions which would minimize secondary reactions. At the present time there is no reported work under such conditions.

In the intense discharge necessary to cause ethane to react at a convenient velocity, the ethylene and acetylene formed are very reactive and polymerize, although they can be readily detected in the gaseous products (25, 30). That they are not always present is indicated by the following gas analysis (159):

SUBSTANCE	VOLUME PER CENT
H_2	48.4
CH_4	13.0
C_2H_6	29.7
C_2H_4	3.5
C_2H_{10}	1.9
C_2H_{12}	3.5

More complete (166) analyses giving the major constituents have been converted to moles of product per 100 moles of ethane reacted and are presented in table 11.

The following equations, based on the data in table 11, account for about 85 per cent of the ethane reacted:

<i>Reaction</i>	<i>Per cent</i>
$C_2H_6 \rightarrow C_2H_4 + H_2$	61
$x(C_2H_4) \rightarrow (C_2H_4)_x$	(50)
$C_2H_6 + C_2H_4 \rightarrow C_4H_{10}$	5
$2C_2H_6 \rightarrow CH_4 + C_3H_8$	15
$C_2H_6 \rightarrow C_2H_2 + 2H_2$	3

It is to be noted that 50 per cent of the ethane which reacts is converted into liquid products, presumably through the polymerization and condensation of ethylene.

During the study of ethane in an ozonizer discharge, an observation was made which has considerable bearing on the reaction mechanism. The reaction products from the discharge tube were passed into two traps

TABLE 11
Products from ethane in an ozonizer discharge

Time of run, minutes	20	20	60	60	60
Products, moles per 100 moles of ethane reacted:					
H ₂	57.4	44.4	61.1	56.8	59.6
CH ₄	16.5	14.1	12.6	14.1	15.0
C ₂ H ₂	4.9	1.0	3.2	4.0	3.2
C ₂ H ₄	4.9	5.5	6.9	5.6	6.1
C ₃ H ₈	16.8	18.0	14.5	15.7	13.2
C ₄ H ₁₀	4.7	5.1	7.0	2.2	5.2
C ₆ ⁺	1.5	3.1	Trace	1.0	1.4
Expansion...	1.02	1.02	1.02	1.06	1.03
Per cent C ₂ H ₆ reacted	15.1	18.1	36.2	46.7	43.5
Per cent C ₂ H ₆ to liquid	6.2	7.2	16.3	23.2	20.4

at -50°C . These traps were connected in series, were as close together as possible, and were in the same Dewar vessel. These traps will be designated 1 and 2 in the order of flow. The exit of trap 2 was connected to two similar traps (3 and 4) by a glass tube 15 cm. long. Traps 3 and 4 were also maintained at -50°C . At the end of the run the amount of liquid in the various traps should decrease in the order $1 > 2 > 3 > 4$. Instead, the order was $1 > 3 > 2 > 4$. Apparently, additional formation of liquid occurs in the relatively long tube connecting traps 2 and 3. This would indicate that some active particle is present which is capable of forming liquid products. At the same time, the particle seems to be fairly stable and has a life of the order of a minute or so, since it has passed through the spirals of both traps 1 and 2 without being deactivated (159).

Since these active particles are intimately connected with the formation

of liquid, it seems quite likely that a careful study of their nature and, if possible, their reactions, would be most fruitful in determining the mechanism of the electrical reactions of hydrocarbons. Certainly an understanding of the fundamentals of these particles would help in the development of these electrical reactions (163).

As has been indicated, the ozonizer discharge causes the formation of liquid products from ethane. A yield of 5.12 cc. of liquid has been obtained from 11.66 g. of ethane. In these experiments, 2 kw.-hr. are required for each gram of liquid. The properties of the liquid are as follows (159):

Color	Reddish yellow
Density			0.862
$n_{\text{Sun}}^{20^\circ}$			1.4900
Molecular weight (cryoscopic in benzene)			467
Bromine			Unsaturated
Per cent C			85.48
Per cent H			13.09
Empirical formula			$\text{C}_n\text{H}_{1.2n}$

In attempting to control the molecular weight of the liquid product, it was found that raising the temperature of the ozonizer from 35° to 70°C. lowered the average molecular weight of the liquid from 467 to 105. This was thought to be due to a lowering of the viscosity of the liquid, permitting it to drain from the discharge more rapidly and thus reducing the amount of secondary action of the discharge on the liquid.

Ethane was studied in the ozonizer, in the semi-corona with both low- and high-frequency excitation, and in the corona discharge with the object of determining the effect of the type of discharge and the discharge conditions on the reaction products from ethane (160). The results of this study are summarized in table 12.

From these data it was concluded that the type of discharge had little effect on the properties of the liquid. When a liquid of low molecular weight is desired, the apparatus should be designed so that the liquid originally formed is removed from the discharge as soon after its formation as possible. When a liquid of high molecular weight is desired, the primary liquid should be left in the discharge to undergo secondary reaction.

To obtain sufficient liquid product to examine in more detail, eleven semi-corona tubes were connected in parallel electrically and in series for gas flow. Traps kept at 0°C. were placed at the exit of each semi-corona tube. The properties of these liquids from the traps were as follows: molecular weight, 170 to 210; density, 0.81 to 0.87; $n_{\text{Sun}}^{20^\circ}$, 1.46 to 1.48. Ethane was converted to 48 per cent of liquid and solid. The energy yields were 0.62 g. of liquid or 0.625 g. of liquid plus solid per kilowatt

hour (161). (In this experiment, the semi-corona center electrode was an aluminum rod 0.3 cm. in diameter. The voltage was 18,000 volts of 60 cycles.)

When ethane was passed through a glow discharge and the reaction products passed over a silver mirror, the mirror remained unchanged. Hydrogen and a resin were isolated as reaction products (14).

TABLE 12
Condensates of low molecular weight from ethane

TYPE OF DISCHARGE	VOLTS	MILLIAMPERES	TEMPERATURE OF ELECTRODES	TEMPERATURE OF TRAPS	MOLECULAR WEIGHT OF LIQUID	$n_D^{20^\circ}$	COLOR	LIQUID
			$^\circ\text{C}$	$^\circ\text{C}$				cc. per hour
Ozonizer.	11600	1	25	25	467	1.4900	Red-brown	0.028
Ozonizer . .	11600	1	70	25	188	1.4642	Slightly yellow	0.011
Ozonizer . .	11600	1	70	-50	184	1.4509	Slightly yellow	0.006
Semi-corona ^(a)	11600	1	70	-50	156	1.4467	Slightly yellow	0.063
Semi-corona ^(b)	11600	1	18	-50	106	1.4503	Slightly yellow	0.100
Semi-corona ^(b)	11600	1	70	-50	105	1.4294	Yellow	0.100
High-frequency semi-corona ^(b)	3000	135	70	-50	95 ^(d)		Red-yellow	0.060
High-frequency semi-corona ^(b)	10000	700	35	-50	109	1.4204	Light yellow	0.100
Corona ^(e)	8000	1		-58	81 and 182 ^(e)		Red-yellow	0.050

(a) Platinum corona wire.

(b) Aluminum corona rod.

(c) Aluminum tube and platinum wire corona.

(d) The lighter of two fractions, the heavier fraction was too insoluble in benzene.

(e) Two fractions separated by vacuum distillation.

4. Propane

By using the same eleven-tube semi-corona discharge apparatus described above, results were obtained which are summarized in table 13 (161).

In the ozonizer, propane was decomposed to the extent of 93 per cent to give 74 per cent of a liquid product whose average composition agreed with C_nH_{2n} (163). Later this study was extended to include careful analysis of the gaseous reaction products (166). These analyses have been

converted to moles of product per 100 moles of propane reacted. The results are given in table 14.

TABLE 13
Action of the semi-corona discharge on propane

	RUN 1	RUN 2
Rate of flow, liters per hour	1 45	0 514
Time, days	9	10
Propane charged, grams	550	218
Composition of gas produced, per cent:		
$H_2 + CH_4$	20 06	45 00
C_2H_6	7 05	9.78
C_3H_8	64.50	41.60
C_4H_{10} + higher	2 39	4 53
Liquid, grams	37.09	63.42
Molecular weight of liquid	115-183	120-160
Density of liquid	0 7424-0 8392	0.70-0.82
$n_{D,20}^{20}$	1 4300-1 4673	1 4352-1 4563
Solid, grams		63.64
Carbon on aluminum rod		1.00
(Grams of liquid per gram of gas charged) $\times 100$	6 7	29 0
(Grams of liquid and solid per gram of gas charged) $\times 100$		58 5
Grams of liquid per kilowatt-hour	0 455	0.480
Grams of liquid and solid per kilowatt-hour		0 985

TABLE 14
Products from propane in the ozonizer discharge

Time, minutes	24.5	24.5	73.5	73.5
Products, moles per 100 moles of propane reacted:				
H_2	48.2	44.0	53.0	55.8
CH_4	18.4	22 3	28.6	27.7
C_2H_6			5.5	4.5
C_3H_8	Trace		6 3	6.8
C_4H_{10}	10.8	3 4	5.9	7.6
C_5H_{12}	39.8	48.0	15.9	17.5
C_6H_{14}	12 3	10.0	7.0	5.7
C_7H_{16}	4.8	3.4	0.8	Trace
Expansion	1 05	1 05	1.10	1.11
Per cent C_3H_8 reacted	12 3	13.3	36.0	40.4
Per cent C_3H_8 to liquid	2.7	3 1	18.1	20.6

The following reaction scheme is proposed to account for these products. As proposed, these reactions can account for about 85 per cent of the re-

acted propane. The reactions which are proposed as the primary reactions are separated from those thought to be secondary reactions. It will be noted that the ratio of the reactions to one another changes as the reaction time is increased from 24.5 min. to 73.5 min.

REACTIONS	PER CENT OF REACTION IN REACTION TIME OF	
	24.5 min	73.5 min.
Primary reactions:		
$C_3H_8 \rightarrow C_3H_6 + H_2$	46	49
$C_3H_8 \rightarrow C_2H_4 + CH_4$	16	27
$2C_3H_8 \rightarrow C_4H_{10} + C_2H_6$	11	7
$2C_3H_8 \rightarrow C_6H_{12} + CH_4$	4	1
Secondary reactions:		
$nC_3H_8 \rightarrow (C_3H_8)_n$	2	32
$mC_2H_4 \rightarrow (C_2H_4)_m$	16	16
$C_2H_4 \rightarrow C_2H_2 + H_2$	0	5

The most important primary reaction is the dehydrogenation of propane to propene, which may be isolated as such or, if the action of the discharge continues, secondary reactions involving propene occur. These are indicated as polymerization in the table.

The second reaction of importance is the demethanation of propane, forming methane and ethylene, with the ethylene undergoing secondary reactions, indicated as polymerization. It should be noted that this interpretation of these data indicates that ethylene, under these conditions, is more susceptible to reaction than propene.

The above two reactions, dehydrogenation and demethanation, are also the main thermal reactions of propane. The ratio of the two reactions is quite different; in thermal reactions the ratio of dehydrogenation to demethanation is 0.8 (227), while in the ozonizer experiment above it is about 2.

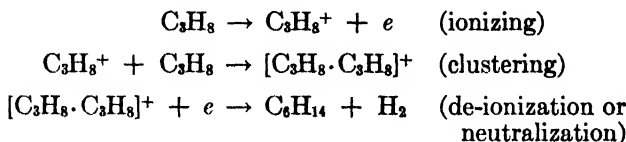
The reactions proposed to account for butane and pentane are of considerable theoretical interest, although they are of minor importance in accounting for the amount of propane reacted. It seems quite possible that activated propane molecules collide and form an association product that is stable for appreciable periods of time, i.e., long enough for a rearrangement of the valence forces within the molecule.



It should be noted in passing that increased pressure should favor this type of reaction to the detriment of the dehydrogenation and demethanation.

If the valence forces within this activated complex do not rearrange, it reverts to propane; if they do rearrange, then it breaks down into butane and ethane or pentane plus methane. It is quite possible that the rearrangement of the valence forces could extend so far that appreciable amounts of isobutane and isopentane are formed. Further, it is quite possible that the complex could decompose to give hexanes and hydrogen, although it is not possible to decide from the data available.

This type of reaction has been proposed to account for the reactions of hydrocarbons caused by alpha-particles and has been extended to include the electrical reactions (162, 163). The theory has been called the "ion cluster" theory and in the case of propane would apply as follows:



This theory is a very useful one. It seems to the present authors that it is possible of even broader application than to ions. As we have indicated in the discussion above, the clustering or at least association between molecules with either or both activated, is a useful concept. In the ion cluster theory, the charge on the ion gives a physical reason for expecting association, while in the activated molecule no such obvious reason is apparent. We see no immediately apparent reason why two saturated molecules with a certain combined energy of activation should not be able to form an unstable molecule within which valence changes could occur before dissociation without ionization occurring at any time. The dissociation of the rearranged product gives two new paraffin molecules.

5. Butanes

Using the eleven-tube semi-corona discharge apparatus described, the results summarized in table 15 were obtained (161).

The same butane mixture has also been studied in an ozonizer system consisting of twelve discharge vessels connected in parallel as to gas flow and electrically. The applied voltage was 20,000 volts of 60 cycles. The run lasted 659 hr., during which time 5420 g. of butane was charged and 1041 cc. (833 g.) of liquid was produced. The liquid product boiled from 0°C. at 740 mm. to 230°C. at 10⁻⁴ mm. Careful fractionating of the liquid gave only one fraction (8.0 g.), which had the properties of an octane containing 22 per cent of octene (162). This (833 g.) is the largest amount of liquid so far reported as produced from a hydrocarbon gas by electrical means.

Later (166) the gases formed in the treatment of a mixture containing 75 per cent of *n*-butane and 25 per cent of isobutane in an ozonizer were analyzed. The time of treatment was 24.5 min. We have calculated the analyses to moles of product per 100 moles of butanes reacted (table 16).

TABLE 15
*Semi-corona discharge in butane**

Gas flow, liters per hour	0.60
Time, days	5.7
Gas used, grams	192
Volts	18,000
Liquid yield, grams	71.1
Molecular weight of liquid	110-140
Density of liquid	0.74-0.78
$n_{\text{Sun}}^{20^\circ}$	1.43-1.45
Solid on glass wall, grams	70.0
Carbon, grams	1.0
Yield, (grams of liquid per gram of gas) $\times 100$	37
Yield, (grams of liquid and solid per gram of gas) $\times 100$	73.5
Grams of liquid per kilowatt-hour	1.09
Grams of liquid and solid per kilowatt-hour	2.23

* The butane used in this experiment was 75 per cent *n*-butane and 25 per cent isobutane (private communication).

TABLE 16
Products from mixed butanes in the ozonizer

Products, moles per 100 moles of butanes reacted:	
H ₂	16.6
CH ₄	11.2
C ₂ H ₄	1.2
C ₂ H ₆	5.3
C ₃ H ₆	19.3
C ₃ H ₈	51.5
C ₄ H ₆	5.3
C ₄ H ₁₀	1.5
Expansion	1.05
Per cent C ₄ H ₁₀ reacted	34.3
Per cent C ₄ H ₁₀ to liquid	11.3

We have not been able to set up equations which will explain these results. The original workers prefer to regard the data as preliminary, since they were unable to separate closely the propane and isobutane by their method of fractionation. They do make the interesting observation that isobutane seems to be formed in the reaction.

From a knowledge of the thermal reactions of butane and by analogy

with the propane reactions we think that the following equations will express the primary reactions of the butanes. No attempt is made to estimate the relative importance of the various reactions.



It is quite possible that the isomerization reaction can be explained adequately by the equation:



using the intermediate activated complex device employed with propane. In the case of propane, the complex could decompose back to propane and not be noticed, but in the case of butane, it is quite possible for a rearrangement to occur and the product decompose into isomerized butane, either or both contributing molecules being isomerized.

Normal butane in the glow discharge gives fragments (radicals) that remove lead and antimony mirrors. The reaction products were not identified (213).

6. Isobutane

It has been stated (166) that isobutane in the ozonizer "did not show appreciable differences compared with its isomer". Presumably this statement applies only to the rate of the reaction, for no analysis of the reaction products is given.

Experience with the thermal reactions of isobutane leads one to expect it to give different ratios of the dehydrogenation and demethanation reactions compared to *n*-butane, even though the overall reaction rates are of the same order of magnitude.

7. *n*-Pentane

At "reduced pressure" in an ozonizer, *n*-pentane gives a decane, a decene, and a yellowish brown compound having the empirical formula $\text{C}_{10}\text{H}_{18}$ (179). An analysis of the gas produced is not available.

In the glow discharge (213) fragments (radicals) are produced that re-

moved lead and antimony mirrors. The other products of the reaction have not been reported. The spectrograms made from the light emitted by the electrodeless discharge in *n*-pentane give lines which are due to to hydrogen atoms and CH radicals (11). A solid brown deposit formed in the discharge tube.

Table 17 gives an analysis of the gas formed from *n*-pentane in the glow discharge.

TABLE 17
Paraffinic hydrocarbons in the glow discharge

HYDROCARBONS	ANALYSIS OF GAS IN VOLUME PER CENT				dp/dt	W
	H ₂	Acetylenes	Olefins	Paraffins		
	per cent	per cent	per cent	per cent		
<i>n</i> -Pentane	60.2	10.0	17.7	12.1	60	1.21
<i>n</i> -Heptane	46.0	9.7	26.9	17.5	101	0.35
<i>n</i> -Octane	48.9	13.8	16.6	20.8	105	
2,2,4-Trimethylpentane	53.2	3.5	27.1	16.2	97	1.32
2,7-Dimethyloctane	56.6	15.1	15.5	12.9	118	0.62
<i>n</i> -Docosane	55.7	11.2	25.7	7.4	155	1.54

dp/dt = rate of gas formation, in cubic centimeters per milliamperere per second $\times 10^4$.

W = rate of solid formation, in grams per milliamperere per second $\times 10^4$.

8. Isopentane

In the ozonizer, isopentane gives a gas containing hydrogen and methane along with 5 per cent of unsaturated hydrocarbons. Fractionation of the liquid product gave a liquid boiling at 100–110°C. and having a molecular weight of 118.6. This fraction was thought to contain an octane (177).

Isopentane has also been studied in an ozonizer in the presence of ammonia. The products consisted of olefins and a base thought to be a hexenylamine (178).

9. *n*-Hexane

Hexane gave a gas containing 40 per cent of hydrogen, 58 per cent of saturated hydrocarbons, and 2 per cent of unsaturated hydrocarbons when treated in an ozonizer. The liquid product formed simultaneously was separated by steam distillation into a colorless, mobile liquid and a yellowish, thick, clear mass. Fractions of the volatile liquid separated by distillation contained from 0.19 to 4.32 per cent of oxygen. One of the fractions had a vapor density corresponding to a heptane (177).

It was observed that hexane was more stable than isopentane under comparable conditions (177).

By conducting similar experiments at reduced pressure, dodecane ($C_{12}H_{26}$), a colorless liquid having the formula $(C_6H_{12})_2$, and a yellowish brown product ($C_{36}H_{64}$) were observed (179).

When a high-frequency discharge was used to excite the ozonizer, hexane at the boiling point gave a gas having the following composition: 43 per cent H_2 ; 12 per cent CH_4 ; 6 per cent C_2H_6 ; 21 per cent C_2H_4 ; 11 per cent C_3H_6 ; 6 per cent C_3H_8 . In addition to this, a $-70^\circ C.$ trap in the exit line contained liquefied gas which analyzed as 70 per cent C_3H_8 and 30 per cent C_3H_6 . The residual liquid contained 5 per cent of 1-hexene and a liquid boiling at $45-120^\circ C.$ at 15 mm., besides the unchanged hexane. This higher boiling liquid contained a small amount of olefinic hydrocarbons. The olefinic constituents were saturated by hydrogenation. The fraction boiling above $100^\circ C.$ at 15 mm. was judged to be cyclic in nature by its hydrogen content. The cyclic compounds were thought to arise from the bivalent radical $-CH_2CH_2CH_2-$ (222).

The differences between the composition of the gases reported to result from the treatment of hexane are worthy of note. These differences emphasize the fact that all the factors in the electrical treatment are probably not known and not readily controlled. While the frequencies of the discharges are different, it seems that this is not the only difference (*cf.* 56).

10. *n*-Heptane

When nickel electrodes were used in a glow discharge, heptane liberated hydrogen and formed a brownish yellow solid which would slowly absorb oxygen. It was partly soluble in organic solvents and reacted with sulfuric and nitric acids and with bromine (53) (see also table 17).

The spectrum analysis of the glow discharge in heptane indicates the presence of carbon and hydrogen atoms and of CH , C_2 , and H_2 molecules (104, 105, 106).

A spectrometric study of the electrodeless discharge in heptane indicates the presence of C^+ ions as well as CH molecules and carbon and hydrogen atoms (104, 107).

Heptane in the glow discharge gave fragments that removed antimony mirrors (213).

11. 2,2,4-Trimethylpentane

A spectrogram of the light emitted by this hydrocarbon in the electrodeless discharge has been made (11), but none of the details have been reported.

Table 17 gives an analysis of the gas produced from 2,2,4-trimethylpentane in the glow discharge.

12. Higher paraffinic hydrocarbons

A number of the higher hydrocarbons, including some of those already discussed, have been studied in the glow discharge. The studies were made at reduced pressures and the gaseous products analyzed. The results are summarized in table 17. The table clearly indicates (a) the powerful dehydrogenating action of this type of discharge and (b) the formation of unsaturated gaseous products (168, 170).

A comparison of the data on the two octanes, *n*-octane and 2,2,4-trimethylpentane, indicates that the branched-chain isomer reacts more slowly (lower dp/dt) but gives more dehydrogenation and more gaseous olefins, while the straight-chain compound gives more acetylenes and more paraffinic gases (167).

"Liquid paraffins" in the ozonizer lose hydrogen and increase in molecular weight and viscosity (78).

We may now ask what the available data indicate towards the commercial utilization of natural gas and gasoline. First, it must be noted that in cases where the power requirements have been measured they are high compared to the amount of chemical changes produced. With more work, it is certain that the power requirements can be reduced. Before any extensive program is undertaken in this direction, it is advisable that the antiknock quality of some of the products in the gasoline boiling range be determined. This will help to decide a part of the potentialities in this direction.

F. THEORETICAL DISCUSSION

The theory of the various types of silent discharge has been excellently presented recently (96, 172), so there is no reason to repeat it here. It does seem worth while to review a few of the essentials and this can be done most clearly with the glow discharge. Particular attention will be paid to the source of chemical action.

Essentials of the glow discharge

The glow discharge has been studied in sufficient detail with elemental gases so that it is fairly well understood (43, 44, 224, 253). The discharge takes place at pressures from about 0.01 to 20 mm. of mercury, so that particles in the discharge have a much longer mean free path than at atmospheric pressures. A diagram of this type of discharge is given in figure 2 with the characteristic portions of the discharge labeled.

In the cathode glow there exists a region of high positive-ion density due to the electrostatic attraction of the negative electrode (cathode). The luminosity is probably due to excitation of molecules by positive-ion collision.

The negative glow is usually the region of greatest luminosity. In it the electron, positive ion, and probably the excited molecule densities are highest. It is a region of almost constant potential.

The Crookes dark space accounts for most of the potential drop of the discharge, and this is where a large portion of the energy dissipation occurs (10). It contains electrons moving towards the anode and positive ions moving towards the cathode.

The Faraday dark space resembles the Crookes dark space, but the energy dissipation and potential drop are much less.

The positive column is a region of uniform ion density and energy unless the column is striated, in which case the energy and ion density vary with the striations. In both cases the electron energies are less than those obtained in the Crookes dark space. The positive ion and electron densities are about 1/100th as great as the densities in the negative glow.

In the anode glow the electron energy is a little higher than in the positive column.

The properties of the individual zones of the discharge and the changes occurring will be discussed, starting with an electron close to the cathode. (The source of the electron at this point will be dealt with later.) Owing to the electrostatic repulsion of the cathode, the electron moves into the Crookes dark space, i.e., the region of greatest potential drop. Owing to this potential the electron is accelerated, its final velocity depending on the potential drop and the pressure in the discharge vessel, i.e., the number of molecules in its path. During this acceleration three types of collisions with molecules occur: (1) Elastic collisions; no energy lost or gained. (2) Ionizing collisions; a positive ion and electron result. (3) Activating collisions; the quantum state of a molecule is raised. This energy may in turn be converted into vibrational energy.

Methods for accurately determining the magnitude of each of these processes are not available. It is known that each electron leaving the cathode forms from fifty to one hundred secondary electrons by ionizing collisions (55, 168). The energy required for an ionizing collision is usually greater than that for an activating collision. Therefore each electron leaving the cathode will undergo many activating collisions. (Only ionized and activated molecules have the energy necessary for chemical reaction, so the elastic collisions may be neglected.

Following the electron through the discharge, we have just shown that it receives most of its energy in the Crookes dark space. In the negative glow the effects of this energy are made manifest, as these electrons continue the loss of this energy in collisions. In this region the electrons must complete the process of forming enough positive ions to maintain the discharge.

Since we are concerned with the chemical action of the discharge, we can regard the rest of the discharge as a diffusion of electrons towards the anode, where they are neutralized, and a diffusion of positive ions towards the cathode.

Consider now the positive ions, which are formed mainly in the negative glow and the Crookes dark space. These ions will tend to move towards the cathode, owing to electrostatic attraction. Even in the Crookes dark space region where this action is greatest, it is quite improbable that these ions will obtain sufficient kinetic energy to cause the activation of other molecules by collisions unless the pressure is low (0.1 mm. or less). The ions do not obtain this energy, (1) because they are heavy when compared to electrons and (2) because of their large collision area it is more probable that they will lose what excess energy they gain, by decelerating collisions. In this respect the positive ions are quite different from electrons (cf. 198).

Eventually most of the positive ions reach the cathode and are discharged. In this discharging process many of the positive ions cause the liberation of an electron from the cathode surface. This is the source of the electron at this point, referred to earlier in this discussion. *For the discharge to be maintained it is essential that every electron leaving the cathode surface form a positive ion which will reach the cathode and liberate another electron.*

When an alternating current is used instead of a direct current in the glow discharge, each electrode alternately acts as the cathode. When the alternations are of the order of 60 cycles per second, the discharge gives the appearance of having a Crookes dark space near each electrode with the positive column in the center. As the frequency is further increased, the length of the Crookes dark space decreases. This in turn means a greater potential gradient in this region, which in turn produces electrons having greater energies (velocities). Since the length of the Crookes dark space is shortened, the total number of electrons formed and accelerated in this region probably decreases. This readily explains the observation that methane, ethylene, and acetylene undergo more profound changes with frequencies of 15×10^6 cycles per second (20 meters wave length), while the total amount of reaction is greater at 6×10^6 cycles per second (48 meters) (65, 204).

Considering the chemical action of the discharge applied to hydrocarbons, we must admit that little is known about the chemical reactions of hydrocarbon ions. We do not know even whether chemical reaction accompanies the neutralization of a hydrocarbon ion at the cathode. A little more is known about the activated molecules from thermal activation in pyrolytic work. There are essential differences between thermal ac-

tivation and electrical (or to be more exact, electronic) activation. In thermal activation, energy must be put into the entire aggregation of molecules and there is only a certain limited probability that the energy will be concentrated in a given bond and cause chemical reaction. In electronic activation the electron collides with the electron which forms a part of the chemical bond, producing activation directly without adding to the translational or rotational energy of the molecule. From this point of view the electrical method is the more efficient method of activating the molecule to cause chemical reaction.

We can sum up the discussion with the statement that accelerated electrons and ions cause the activation of molecules so that chemical reaction may take place at pressures below about 0.1 mm. Above this pressure the ions activate fewer molecules, so that at atmospheric pressure the electron can be regarded as the primary activating agency.

It will be noted from the above discussion that *it is not necessary for a molecule to become ionized to react chemically in the discharge* (73). If we assume that a hydrocarbon ion can be discharged at the cathode without undergoing chemical reaction, then it would seem that only a very minor portion of the reaction product would be due to ions. (The above assumption can be partly justified by noting that the energy of neutralization at the cathode can be used to liberate an electron from the cathode, a process which is essential to the maintenance of the discharge.)

The fundamentals of the glow discharge may be summarized (43) as follows (1) The major part of the chemical reaction takes place in the negative glow. (2) The reaction rate in the negative glow is independent of pressure within the limits 0.2 to 20 mm. (3) The rate of reaction is proportional to the current. (4) Reactions in the negative glow have a zero or negative temperature coefficient. (5) In synthesis (combination of two or more molecules) the rate of reaction is accelerated by the addition of the gas having the higher ionization potential and retarded by the addition of the gas with the lower potential.

In addition to the above theory of the glow discharge, there are several interesting points about the ozonizer discharge which should be noted (cf. 148):

1. If the glass acts as an electrode, then we might well expect some change in the glass. This has been noted in several cases. The action is an erosion of the glass until it assumes the appearance of a ground-glass surface. As the eroding action continues, the glass is finally punctured by the electrical potential and becomes useless for further service (78).

2. When the ozonizer apparatus is supplied with a 60-cycle alternating current, it has been found that the electrical condenser (capacity) characteristic comes into resonance with the inductive part of the circuit causing

high-frequency oscillations (144, 65, 99). It is thus not possible to say what part of the chemical action of the discharge is due to the frequency supplied and what part due to the resonance frequency.

3. Chemical action taking place in the ozonizer apparatus *increases* the electrical conductance of that apparatus. For example, an ozonizer causing chemical reaction in a gas conducts more current than the same apparatus with the reaction space filled with mercury (144).

By studying several hydrocarbons under comparable conditions, relative reaction rates are obtained from the per cent of hydrocarbon reacted in a given time (166) (see table 18).

If we knew the number and energy of the electrons causing the reaction, we would be able to calculate the relative reaction rates in more fundamental terms. The technique for such a determination has not been worked out. Nevertheless this has been approximated by Lind and Schultze (166),

TABLE 18
Relative stability of hydrocarbons in the ozonizer discharge

HYDROCARBON	PER CENT REACTED
CH ₄ . . .	7.9
C ₂ H ₆ .	17.0
C ₃ H ₈ .	12.9
C ₄ H ₁₀ (both isomers).	32.8
C ₂ H ₄	39.6
C ₂ H ₂	65.0
C ₂ H ₂	75.5

using similar data obtained with alpha-particles where the energy and number of activating particles are known. This approximation uses the following quantities: M = number of molecules reacting; N = number of ion pairs (alpha-particles); k = total specific ionization of the reacting molecule; and s = ion-stopping power of the reacting molecule. k and s are obtained in terms of Bragg's equations (41).

It is then assumed that the quantity

$$\frac{M}{N} ks$$

governs the electrical reactions of hydrocarbons.

In the alpha-ray work, M/N and ks are known for several hydrocarbons. For example, M/N for methane is 2 and $ks = 1$, so that

$$\frac{M}{N} ks = 2$$

Now if this quantity $\frac{M}{N} ks$ governs the rate of reactions in the electrical discharge, then the per cent conversion should be proportioned to $\frac{M}{N} ks$ under comparable conditions, i.e.,

$$\text{per cent conversion} = C \frac{M}{N} ks$$

where C is a function of the apparatus, the conditions of the experiment, etc. As these were fixed, C is a constant and should be applicable to all hydrocarbons studied under those fixed conditions. In the case of meth-

TABLE 19

Relative stability of hydrocarbons to the ozonizer discharge compared with the stability to alpha-particles

	CH ₄	C ₂ H ₆	C ₃ H ₈	C ₄ H ₁₀	C ₂ H ₂	C ₆ H ₆	C ₂ H ₄
$\frac{M}{N}$ from alpha-ray work	2	2	2	2	5.5	4.9	19.2
ks	1.0	2.0	3.0	4.0	1.7	2.58	1.4
Per cent conversion (table 18)	7.9	17.0	12.9	32.8	39.6	48.0	75.5
Calculated $\frac{M}{N} ks$ (electrical)	2*	4.5	3.3	8.3	9.9	12.2	19.1
$\frac{M}{N} ks$ (alpha-ray)	2	4	6	8	9.4	12.6	27.7

* This value was used in evaluating C .

ane the per cent conversion is 7.9 per cent under these conditions. Using the above values of M/N and ks for methane we get

$$7.9 = 2C$$

$$C = 3.95$$

We can now use C to calculate $\frac{M}{N} ks$ for the electrical reactions and compare them with the experimentally determined alpha-ray studies. The values are summarized in table 19. With the exception of propane and acetylene the agreement is good. The reaction of acetylene is known to involve some inhibiting factor whose nature is unknown. No explanation can be given for the result with propane.

This work of Lind and Schultze indicates that the fundamentals of the electrical reactions are beginning to be understood. At first sight, it would seem that, by using the total specific ionization, k , every molecule

must become ionized to react. An analogous factor, "the total specific activation", can be introduced and, if the total specific activation is proportional to the total specific ionization, the same relationships still hold. Our contention that ionization is essential to maintain the discharge but is not essential for reaction is unchanged.

IV. REACTIONS IN ARC AND SPARK (DISRUPTIVE) DISCHARGES

"The arc discharge is characterized and distinguished from other types of discharge by its exceptionally low cathode fall of potential and its high current densities" (that is, amperes to thousands of amperes per square centimeter; 172, page 605). The spark is "an unstable and discontinuous occurrence marking the transition from one more or less stable condition of current between electrodes in a gas, to another one" (172, page 408). Thus, in many cases the spark is a precursor of the arc. (For an excellent bibliography and theory of the spark see reference 184.)

The arc and spark are also closely related in the types of reactions that they cause when the discharge takes place in hydrocarbons. In both cases the reactions are those that would be expected if the hydrocarbons were exposed to high temperatures, i.e., 1500°C. and perhaps higher. In dealing with such high temperatures it must be remembered that the reaction zone is restricted and consequently the contact time can be made very short so that the reaction is controlled. While there may be electrical effects on the hydrocarbon in the spark discharge, there is reasonable doubt that there is any such effect in the arc (81).

While the thermal effect is probably the outstanding effect in these discharges, it is noticeable that the electrode material in many cases plays an important rôle in the reaction. This may be due to a catalytic effect, as in the case of iron or nickel, or it may be a definite chemical reaction where the electrodes actually take part in the reaction, as has been noticed in some cases with carbon electrodes. Undoubtedly there are also electrons and ions in these discharges. Just how much they influence the final reaction products cannot be told at this time.

With these effects in mind it is not possible as yet to present any satisfactory mechanism for the reactions taking place.

A. REACTIONS OF HYDROCARBONS IN THE SPARK DISCHARGE

1. *Methane*

It was realized quite early (1798) that electric sparks caused reactions in hydrocarbon gases (115) with the formation of higher boiling products. It was also soon found that the spark also decomposed the hydrocarbons into carbon and hydrogen (60, 115, 209). It is quite possible that acetylene was also present but remained undetected in the large volume of

hydrogen. It remained for Berthelot (23), in his comprehensive studies on acetylene, to show its presence. Table 20 gives a summary of the work that has been done.

TABLE 20
Gaseous paraffins in the spark discharge

HYDROCARBON	REACTION PRODUCTS	REFERENCE
Hydrocarbon gas	H ₂ , C, liquids	(115)
Hydrocarbon gas..	H ₂ , C	(35, 47, 60, 89, 156, 209, 250)
CH ₄	H ₂ , C ₂ H ₂ , C, 80% increase in volume	(23)
CH ₄	12.5% C ₂ H ₂ (50% yield on methane reacted)	(21, 24)
CH ₄	Up to 14% C ₂ H ₂ , 65% H ₂ . Carbon, and liquid and solid hydrocarbons	(236)
CH ₄	5 volumes H ₂ and 1 volume C ₂ H ₂	(93)
CH ₄	H ₂ , C ₂ H ₂	(58)
CH ₄	One or both electrodes metal: H ₂ and C	(182)
	Both electrodes carbon: H ₂ and C ₂ H ₂	
CH ₄	7 volumes H ₂ and 1 volume C ₂ H ₂	(30)
CH ₄	Olefins and acetylenes	(132)
CH ₄	H ₂ , C ₂ H ₂	(3)
C ₂ H ₆	H ₂ , CH ₄ , C ₂ H ₂ , and C ₂ H ₄	(93)
C ₂ H ₆	H ₂ , C ₂ H ₂	(3)
C ₃ H ₈	H ₂ , C ₂ H ₂	(3)
n-C ₄ H ₁₀	H ₂ , C ₂ H ₂	(3)
i-C ₄ H ₁₀	H ₂ , C ₂ H ₂	(3)

TABLE 21
Products formed by treating methane with electric sparks
Volume per cent of products in final gas at pressures indicates

TIME OF SPARKING	OLEFINS			ACETYLENES		
	40 cm	60 cm	70 cm.	40 cm.	60 cm	70 cm.
minutes						
1	1 10	2.05	3 90	7 50	4.85	5 20
2	1 55	3 15	5.35	8 45	7.40	7.75
3	1.90	3 70	5 90	8 90	7.55	7 80
4	2 20	4 05	6 00	9 00	7 00	7.85

By controlling the pressure and the time of sparking (platinum electrodes), the reaction products can be controlled to some extent. This is indicated in table 21.

It will be noted that the higher pressure favors the olefin yield, but is slightly unfavorable to the acetylene yield. For the maximum yield of useful products the higher pressure is definitely indicated (132).

Methane has also been studied using a wide sheet of spark produced

between two flat strips of copper. The methane was passed perpendicular to the spark sheet. The gaseous product contained 14 per cent of acetylene, "large quantities of hydrogen," 2 per cent of ethylene, traces of hydrogen cyanide, and higher hydrocarbons (236).

The liquid product was thought to be mainly dipropargyl ($\text{CH}\equiv\text{CCH}_2\text{CH}_2\text{C}\equiv\text{CH}$) along with small amounts of benzene. The yield of this liquid varied from 0.007 to 0.026 g. per liter of methane.

The tar produced contained naphthalene and acenaphthene along with other substances not identified. The yield of tar varied from 0.01 to 0.051 g. per liter of methane.

In other experiments electrodes of copper, gold, aluminum, or platinum did not seem to alter the course of the reaction, while iron electrodes seemed to favor carbon formation (236).

The use of metal electrodes or one metal electrode and one carbon electrode is said to favor carbon formation, while acetylene formation is favored if both electrodes are carbon (182) when treating methane by sparks.

Methane was treated with sparks of about 50 kilovolts at 100 kilocycles. The yield of acetylene increased with increasing rate of flow and decreased with the reaction time and the rate of decomposition of the methane. The methane reacted varied from 73.7 to 100 per cent and the acetylene yields from 38.4 to 47.3 per cent of the methane reacted. Dilution of the methane with an equal volume of hydrogen or carbon monoxide increased the acetylene yield. Nitrogen changed the yield slightly and carbon dioxide, oxygen, or water vapor decreased the acetylene yield greatly (3).

The physical changes taking place in a spark between nickel or copper electrodes in methane have been observed through a low-powered microscope. The electrical source was a high tension d.c. magneto. When there is no added resistance in the circuit, small incandescent particles pass to the anode, then fly suddenly to the center of the gap and deposit on the cathode. With 5,000 to 100,000 ohms resistance in the circuit, a carbonaceous thread-like deposit builds up on the cathode. This deposit is conducting, so that the discharge takes place from the anode to the end of the deposit. Reversing the polarity of the discharge gradually removes a preformed deposit (128).

2. Ethane

Ethane when passed through 8- to 47-kilovolt sparks between spherical electrodes gave hydrogen, acetylene, methane, and ethylene (93). Ethane when treated with 50-kilovolt 100-kilocycle sparks gave hydrogen and acetylene, along with minor amounts of ethylene and other saturated and unsaturated hydrocarbons. Dilution with hydrogen increased the acetylene yield (3).

3. Propane, *n*-butane, and isobutane

These gases, when treated with 50-kilovolt sparks at 100 kilocycles gave hydrogen and acetylene as the major products in yields of 61.1 to 87.3 per cent of theory. Small amounts of ethylene and other olefins and paraffins were simultaneously formed (3).

4. *n*-Pentane

Sparks passed between platinum electrodes immersed in boiling *n*-pentane gave a gas containing hydrogen, methane, and probably ethane, ethylene, and acetylene. No carbon was deposited (254).

5. Ethylene

Hydrocarbons from ethyl alcohol and from ethyl ether, presumably containing ethylene, gave oily droplets on sparking (87).

The uncontrolled action of sparks on ethylene is to form carbon and hydrogen. This has been observed with platinum (60) and copper elec-

TABLE 22
Products from ethylene upon passage through a 33,000-volt spark

ETHYLENE	ETHYLENE REACTED	VOLUME PER CENT COMPOSITION OF GASEOUS PRODUCTS		
		C ₂ H ₂	H ₂	CH ₄
<i>cc per hour</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
754	85.2	28.8	67.8	3.4
3115	58.0	33.4	64.8	1.8

trodes (182, 209). The carbon-forming action of aluminum electrodes is intense compared with gold electrodes (189).

If the action of the sparks is controlled, the gas produced contains 14 per cent of acetylene and 86 per cent of hydrogen (22). It has also been noticed that under comparable conditions ethylene reacts more slowly than acetylene. The ethylene forms a small amount of a liquid product that has the odor of turpentine or petroleum (269).

A mixture of equal volumes of ethylene and argon gave hydrogen and a small quantity of methane as the gaseous reaction products (34). The effect of the argon is not clear.

A more quantitative study (93), using 33,000 volts across the spark gap, indicates that the extent of decomposition was proportional to the time of contact and that some of the acetylene formed originally was decomposed into carbon and hydrogen. Table 22 indicates the type of results obtained (93).

It is claimed (121) that a spark discharge passed between copper elec-

trodes 8 cm. apart with the current adjusted so that the discharge consumed 60 watts will give products containing 9.1 per cent of propene, 1.4 per cent of butene, and 7.7 per cent of butadiene when ethylene is passed through the discharge at 20 liters per hour.

Ethylene treated with 50-kilovolt 100-kilocycle sparks gave mainly acetylene and hydrogen (3).

6. *Propene, 1-butene, and 2-methylpropene*

When treated in 50-kilovolt 100-kilocycle sparks these hydrocarbons gave acetylene and hydrogen as the main products, with small amounts of ethylene and other olefin and paraffin hydrocarbons. When the reacting gases were diluted with hydrogen, the amount of ethylene was increased (3).

7. *Pentenes*

Sparks passed between platinum electrodes dipping in boiling "amylene" caused the separation of small amounts of carbon and the evolution of a gas containing hydrogen, methane, acetylene, ethylene, and probably ethane (254).

8. *Acetylene*

Acetylene gives primarily the elements in the spark discharge (20, 26, 33, 88, 194, 268), although small amounts of liquid and polyacetylenes have been detected (28). The tendency for acetylene to revert to carbon and hydrogen can be vividly illustrated by the observation that an electric spark in acetylene at 3 atmospheres pressure caused a violent explosion (48).

9. *Butadiyne*

When a spark was passed through gaseous butadiyne, decomposition occurred with production of a dazzling white light. The main decomposition was to carbon and hydrogen, although there was a 4.5 per cent contraction in volume, indicating that some hydrocarbon, presumably methane, had been formed (243).

10. *Benzene*

A spark between platinum wires dipped in benzene gave carbon and a gas containing 42 to 43 per cent of acetylene and 57 to 58 per cent of hydrogen (64, 80). Carbon electrodes under somewhat similar conditions gave a gas containing 44 per cent of hydrogen, 53 per cent of methane, and 3 per cent of acetylene and other unsaturated hydrocarbons. A liquid residue was formed which boiled above 250°C. and had an average

molecular weight of 300 (88). Whether the difference in the electrodes is the sole cause of the observed differences in gas composition in the two experiments is not known.

11. Toluene

With platinum electrodes a spark discharge in toluene gave carbon and a gas containing 76 to 77 per cent of hydrogen (64).

B. ARC DISCHARGE

1. Methane

A carbon arc in a closed vessel filled with methane gave, after 1 hr. of arcing, 9 per cent of acetylene, 2 per cent of methane, and 89 per cent of hydrogen. A gas of the same final composition was obtained when the original gas was hydrogen, clearly indicating that the carbon of the electrodes is actually part of the reaction system (37, 38).

When methane is passed through the arc, acetylene forms in yields of 6 to 12 per cent of the methane passed or 26 to 32 per cent of the methane reacted. The rest of the methane is converted into a fine soot-like carbon and hydrogen. Dilution of the methane with hydrogen tends to reduce the formation of carbon. With a mixture of 1 volume of methane and 2 volumes of hydrogen, the arc treatment gave acetylene equivalent to 51 per cent of the methane charged or 68 per cent of the methane reacted, in a single pass (91, 153).

Instead of diluting the reacting gas with hydrogen, a similar effect can be obtained with a high-tension arc at reduced pressures (39, 40). At pressures of 100 to 200 mm. of mercury the exit gas contained 12 to 14 per cent of acetylene with an energy consumption of 12 kw.-hr. per cubic meter of acetylene produced. The effect of either hydrogen or reduced pressure is to decrease the amount of carbon formed, so that the reaction is more selective.

A study of arcs with different intensities (energy consumption) indicated that an arc consuming up to 150 watts promotes cracking to acetylene, while arcs of higher energy promote the formation of carbon and hydrogen (39, 193). It seems likely that factors other than energy consumption, e.g., electrode material, size, cooling, etc., are also pertinent.

Butadiyne has been isolated from the gases produced by the arc in methane. There is also some evidence that allene, methylacetylene, and butadiene are formed (248).

It is to be noted that heating the chamber enclosing the arc promotes the formation of more carbon and hydrogen, probably owing to the secondary reaction of acetylene. For this reason it is desirable to cool the arc vessel when acetylene is the product sought (217).

Electrodes of iron and nickel have also been used in the study of methane in the arc discharge. At 110 volts and 4 to 5 amperes d.c. the rate of decomposition of the methane and the concentration of acetylene in the gas produced were higher with carbon electrodes than with iron or nickel electrodes, but the yield of acetylene on the basis of the methane reacted was the same in all cases. At a contact time of 0.002 sec. in the carbon arc, 18 per cent of the methane reacted. The acetylene yield was 9 per cent of the original methane or 50 per cent of the methane reacted. A mixture of methane and hydrogen increased the acetylene yield (146).

2. Ethane

Ethane gave ethylene and acetylene in nearly equal amounts when treated in the electric arc (126). Low gas velocities through the arc caused the formation of carbon and hydrogen.

3. Ethylene

Passing an arc between platinum electrodes in ethylene until the maximum expansion occurred (about 10 min.), produced hydrogen as the only gaseous product. Presumably the other product was carbon (47).

4. Acetylene

Using carbon electrodes, an arc in acetylene gave predominantly carbon and hydrogen, along with a small amount of methane and enough naphthalene so that it could be recognized by its odor (37, 38).

5. Pinene

Pinene gave ethylene, acetylene, and isoprene, in addition to unidentified substances (260).

6. Liquid paraffin hydrocarbons

When hexane vapor was studied with a 3- to 4.5-ampere, 60- to 80-volt arc between copper or iron electrodes a gas was produced which analyzed as follows: paraffins, 4 per cent; olefins, 0.5 per cent; acetylene, 1 per cent; hydrogen, 94.5 per cent (116).

When the conditions were altered so that the electrodes were still in the vapor space but were kept wet by the refluxing hydrocarbon, the gas contained 10 to 16 per cent of olefins and 4.8 to 5.6 per cent of acetylene. The olefins were not identified, except that the presence of butadiene was proved by isolating the solid tetrabromide. In addition to hexane, the hydrocarbons heptane, octane, nonane, and decane were studied with both iron and copper electrodes. The higher boiling hydrocarbons seemed to favor slightly higher yields of olefins, although the trend is slight. The

iron electrodes gave more carbon deposits than the copper electrodes, but no significant effect was noted on the olefin or acetylene concentration in the gas. The carbonaceous deposit formed in the presence of iron contained 20.1 per cent of iron; the analogous deposit with copper electrodes contained 0.59 per cent of copper. The deposit on the iron electrode was bright and lustrous, while the copper electrode deposit was dull (116).

When the experimental conditions were further altered so that the arc was immersed in the liquid, it was found that hexane, heptane, and decane gave a gas that contained 5 to 16 per cent of olefins and 13 to 15 per cent of acetylene. It is thus apparent that the method of conducting the arcing of the hydrocarbon greatly affects the composition of the gas produced (116).

7. Benzene

Benzene has a distinct tendency to form carbon and hydrogen in the arc; at the same time there are usually formed liquid products boiling above benzene. The gas formed is 80 to 90 per cent hydrogen (202).

To study the reaction more carefully, 17 liters of benzene were treated in the carbon arc and the products passed through traps at room temperature and at -36°C . Under these conditions there were 8 liters of product in the arc vessel, 540 cc. in the trap at room temperature, and 320 cc. in the trap at -36°C . The residue in the arc vessel was 95 per cent benzene and 5 per cent higher boiling material. From the higher boiling material phenylacetylene, diphenyl, and anthracene were isolated. Butadiyne was recovered from the -36°C . trap. In a separate experiment 194 g. of carbon was obtained during the treatment of 780 g. of benzene (195).

When the arc is immersed in liquid benzene under some conditions, the carbon formed builds a bridge across the arc and shortcircuits it. Metal electrodes were found to prevent this, because the carbon was kept in suspension. With metal electrodes, acetylene and ethylene were formed as well as hydrogen (261).

An arc between iron or copper electrodes that were in the vapor but were kept wet by refluxing benzene gave a gas that contained 0.2 to 0.3 per cent of olefins (116). No other analyses are given.

8. Toluene

Toluene gave a gas containing 2 to 2.5 per cent of olefins when subjected to an arc between iron or copper electrodes that were in the vapor but were kept wet by refluxing toluene (116).

9. Naphthalene

Only carbon and hydrogen have been reported as products from naphthalene in the arc (171).

V. COMMERCIAL PROCESSES

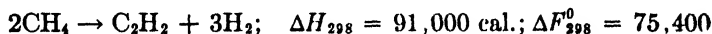
From the literature available it is not possible to give complete details on the commercial processes for the electrical treatment of hydrocarbons. Neither is it possible to say just which of the proposed processes have actually been commercialized. Nevertheless it is instructive to review briefly the processes which have been proposed, along with such pertinent characteristics as may be obtained from the issued patents. We fully realize that the information obtained from patents may not always be reliable.

A. ACETYLENE PRODUCTION

The current method of producing acetylene is from calcium carbide. The main reaction in preparing the carbide is



It is also possible to prepare acetylene from methane (or natural gas) by the reaction



Thus the theoretical energy requirement for the carbide reaction is more than 15 per cent greater than that of the methane reaction. Converting the ΔH requirement into electrical units, the carbide reaction requires 5.6 kw.-hr. per cubic meter of acetylene, while the methane reaction requires 4.7 kw.-hr. per cubic meter.

In actual practice the energy requirements are greater. It has been stated that 8.5 kg. of 310 liters per kilogram carbide can be produced per kilowatt day (259). This indicates a practical energy requirement of 9.1 kw.-hr. per cubic meter. Further, to get the power requirement this low, very large furnaces (15,000 to 25,000 kilowatts) must be used. On smaller furnaces the energy requirements may be as high as 15 to 18 kw.-hr. per cubic meter of acetylene.

The corresponding practical energy requirements for acetylene from methane are not known. From the information available, however, it seems that the following factors would tend to favor the production of acetylene from methane: (1) The theoretical or limiting energy requirement for the methane reaction is lower. (2) The raw material, methane, is cheaper in many localities than lime and coke. (3) Gaseous methane is readily transported in pipe lines to the centers of "cheap" electrical power.

The factor favoring the carbide process is that the acetylene produced is comparatively pure, while that from methane is mixed with hydrogen and unconverted methane.

Not only has methane been used as a source of acetylene in the arc, but also various hydrocarbon mixtures from both petroleum and coal. In all cases carbon, as a more or less light soot, is formed as a by-product. Much of the study has been in the direction of minimizing this carbon formation and devising mechanical schemes for operating the process without the soot plugging the necessary pipes and valves.

In the case of petroleum products, graphite electrodes are much better than copper or iron for making high yields of acetylene and reducing the soot formation (83).

In the Claude process (173) the liquid hydrocarbon is atomized into the arc through one of the electrodes. The soot is removed by an oil scrubber and the acetylene separated from the hydrogen and other gases by compressing to 10 atmospheres and extracting with water in which the acetylene is considerably more soluble than the other constituents.

It is stated that 1 cubic meter of acetylene requires 2.2 kg. of raw oil, 14 kw.-hr. for the furnace, 1.5 kw.-hr. of pumping energy, and 3.4 g. of electrodes. At the same time about 1.4 cubic meters of hydrogen is produced (173; also 77, 82, 99, 171, 193, 216).

In the case of a petroleum fraction boiling in the hexane range it was found that at the beginning of the arc treatment the gas contained 18 per cent of acetylene. After a short time of operation the hexane became heavily laden with soot and the acetylene in the gas increased to 33 to 34 per cent. Removal of the soot by filtration and again treating the hexane in the arc gave 18 per cent of acetylene. Thus the soot seems to be intimately related to the acetylene-forming process. Lamp black or coal dust added to the hexane produced no effect, but soot produced in one run could be added to fresh hexane and the 33 to 34 per cent of acetylene realized immediately (58).

In view of the high hydrogen yields in the arc cracking of methane or natural gas, this method has been proposed as a method of making hydrogen. Using an arc of 400 to 500 volts and 7 to 12 amperes, the hydrogen yield was 88 per cent of the theoretical in one pass through the arc (67, 124, 267).

The patents pertinent to making acetylene from hydrocarbon materials by treatment in the electric arc deal mostly with types of apparatus and operative details (16, 17, 70, 71, 98, 100, 103, 119, 123, 131, 150, 186, 200, 218, 232, 233). The main generalization is that it is desirable to dilute the reacting gas or vapor. Hydrogen is the diluent most frequently mentioned (41, 46, 72, 123, 142, 155, 170, 232), although carbon monoxide, carbon dioxide, and steam have been claimed.

It has been proposed that acetylene be made from hydrogen plus carbon monoxide or carbon dioxide in the arc (15, 57).

The carbon that is regarded as a nuisance in the preparation of acetylene is, in many cases, so light, fluffy, and black that it has value as a black pigment and as the carbon black component of rubber articles. Special techniques have been designed for its manufacture from hydrocarbons in the arc (103, 130).

When anthracene oil was subjected to impulse discharges (the spark discharge from an electrical condenser) a gas containing 22 to 30 per cent of acetylene was obtained. The energy requirement was 11 to 12 kw.-hr. per cubic meter and was independent of condenser capacity in the range 5 micromicrofarads to 0.25 microfarad (154, 218).

It is claimed (125) that hydrogen can be activated in the arc and that when the activated hydrogen is brought in contact with hydrocarbons, olefins and acetylene are formed. Or, if the hydrocarbon is ethylbenzene, phenylacetylene is formed (120).

The electric spark has also been proposed as a means of converting coal gas into acetylene on a commercial scale (100).

B. LUBRICATING OILS

The polymerizing and dehydrogenating action of the silent discharge have been used advantageously to produce and improve lubricating oils (5, 6, 7, 9, 18, 36, 45, 49, 68, 79, 90, 94, 101, 102, 112, 118, 122, 126, 136, 139, 145, 196, 197, 201, 203, 207a, 210, 212, 215, 230, 235, 237, 258, 264, 265, 271, 272, 273). A commercial process is known as "Elektronization" or "Voltolization".

The apparatus used for this treatment consists of a series of aluminum electrodes in the form of flat plates about 80 cm. square and 1 mm. thick. The electrodes are built up as a bank of plates of alternating polarity until a total area of about 190 square meters is obtained; each plate or electrode is separated and insulated from its adjacent plate by glazed pasteboard 2 mm. thick. The pasteboard extends beyond the edges of the plate electrodes to prevent sparking from plate to plate.

The reaction vessel consists of a horizontal cylinder which contains four of the electrode banks described above, to give a total plate area of 750 square meters (8050 sq. ft.). The electrodes are mounted so they can be rotated about a central shaft at 1 r.p.m. During this rotation the electrodes dip into the oil contained in the cylinder (one-quarter full of oil). At the same time appropriately placed buckets dip into the oil and gradually pour the oil over the electrodes. In this way the oil on the electrodes is continually replaced so that overexposure to the discharge does not occur.

The reaction vessel is equipped with a few coils of pipe which are used at the start to heat the charge up to 80°C. and during operation are used

as cooling pipes to keep the temperature down to 80°C. Also attached to the reaction vessel is a small suction pump which maintains the pressure in the vessel at about 60 mm. of mercury (Absolute).

The electrical energy is supplied by a special alternator producing 500-cycle current. This is stepped up by transformers to give the desired voltage. Most of the published work indicates that this voltage is 4300 to 4600 (126, 203), although a photograph of part of the equipment in one of the plants shows a vessel labelled 50,000 volts (49). For the type of equipment described above, the 50,000 volts would be much too high.

In use the apparatus is filled one-quarter full of the oil to be treated (about 2000 gallons) and the air in the vessel is replaced by hydrogen at 60 to 65 mm. of mercury pressure (Absolute). When the current is turned on, the vessel is filled with light from the discharge. The action of the discharge causes dehydrogenation of the oil and the hydrogen must be pumped off. The dehydrogenated oil polymerizes to form larger molecules. This dehydrogenation and polymerization action of the discharge is analogous to the reactions which have already been discussed in connection with the pure hydrocarbons. The net result of the action of the discharge is to increase the viscosity of the oil. It is possible to continue the action of the discharge until the oil coagulates and forms a gel.

When a pure mineral oil is treated in the discharge, the rate of reaction is usually too slow to be economical (203). On the other hand, the fatty oils (fish, animal, and vegetable) polymerize much faster. As a result, it is customary to start with a fatty oil or a fatty oil-mineral oil blend and treat this. The treated product is diluted with mineral oil and re-treated until the final product contains about 15 per cent of the fatty oil. The properties of the oil so produced are as follows (8):

Specific gravity at 15°C.	0.925
Viscosity at 100°C.	2000 centipoises
Flash point (open cup)	225°C.
Conradson carbon	0.4 per cent
Pour point	-5°C.
Acid number	1
Saponification number	90
Ash	0

It is indicated that this oil is to be blended with mineral oils up to 15 per cent. The following changes take place when this is done: (1) the viscosity increases; (2) the viscosity-temperature coefficient is decreased; (3) the pour point is lowered; (4) the sludge-forming tendency of the oil is decreased; and (5) the tendency to form emulsions with water is increased.

The history of this process has been a romantic and turbulent one. It began in 1904, when de Hemptinne (112) found that hydrogen could be

activated in the silent electric discharges so that it would hydrogenate oleic acid, converting it into stearic acid. With both hydrogen and oleic acid in the discharge it was found that the oleic acid polymerized, giving viscous oils, instead of hydrogenating to stearic acid. Later it was found that the unsaturated glycerides could be polymerized, giving viscous oils suitable for lubricants. The use of the process for commercial purposes was begun in 1907 (139), when the Société Anonyme Elektrion was formed in Ghent, Belgium. This plant was in operation in 1914 and was commandeered by the Germans when they occupied Belgium. Apparently the Germans operated the Ghent plant throughout the war and also started a plant in Germany with the formation of the Deutsche Elektrion-Oel Gesellschaft. At the end of the war the Germans obtained the right to manufacture these products in Germany but were forced to abandon the name "Elektrion", which was the property of the Belgian company. The Germans coined the name "Voltöl", renaming their company the Deutsche Voltöl Gesellschaft.

Just what mineral oils are most desirable for electrical treatment is not disclosed. It has been found (225) that the oil produced by the alkylation of naphthalene and tetralin by ethylene in the presence of aluminum chloride can be electrically treated. The viscosity is increased, but from the data given it would seem that the viscosity-temperature coefficient has not been decreased.

Other work (207) indicates that the type of original hydrocarbon has a profound influence on the final viscosity characteristics. Several pure compounds have been studied in an ozonizer for 6 hr., using 7500 volts, 1000 cycles, and 10 to 20 milliamperes. The results are given in table 23.

These data indicate that the straight-chain olefins give better products and higher yields than the cyclic and aromatic hydrocarbons. This may not be the whole story, for it has been claimed that non-paraffinic extracts produced by solvent extraction of petroleum oils give suitable "Voltöls" (234). This may arise from the fact that broadening the boiling range of a lubricating oil is known to increase its viscosity index. Where the "Voltöl" is used as a blending oil (as it usually is), it is entirely possible that the viscosity index of an oil could be apparently increased by a "Voltöl" with a very low viscosity index and the entire effect may be due to a broadening of the boiling range. Too little is known about the theory of lubricating oils and viscosity index to offer this as anything more than a possibility.

The optimum conditions for the "Voltölization" process have been studied and the results on a laboratory scale published (249). A cracked kerosene having a kinematic viscosity of 2.88 at 20°C., and 1.68 at 50°C. was used. The experiments were made in an ozonizer that could be oper-

TABLE 23
Viscosity characteristics of "Vollolized" pure hydrocarbons

HYDROCARBON	KINEMATIC VISCOSITY			VISCOSITY INDEX*	YIELD	BOILING POINT ABOVE
	At 20°C.	At 50°C.	At 100°C.			
					per cent	°C.
Caprylene	17 2	6 3	2 0		50	200
Cetene	142 0	34 4	8 4	123	60	Original
n-Dodecane	11 43	5 00	2 02		23	Original
1,2,4-Trimethylcyclohexane	1928	128	12 7	-56	28	200
Decalin	13071	399	20 7	-220	26	200
Cumene		1143	29 0	-470	17	200
Methylnaphthalene		5000	55 3		15	200

* We have used a plot of the viscosity-temperature curve to estimate the viscosity index

TABLE 24
Effect of conditions of "Vollolization" on the viscosity of a cracked kerosene

TIME	CONDITIONS			KINEMATIC VISCOSITY	
	Frequency	Voltage	Current	At 20°C	At 50°C.
hours		kilovolts	milliamperes		
2	350	12 2	10	6 36	3 03
4	350	12 2	10	14 0	5 30
6	350	12 2	10	64 4	17 2
2	500	7 25	5 5	4 02	2 08
4	500	7 25	5 5	5 68	2 80
2	500	11 4	10	7 24	3.88
4	500	11 4	10	20 2	7 2
6	500	11 4	10	66 0	18 0
10	500	11 4	10	178 7	22 0
2	750	9 0	10	5.64	2.82
4	750	9 0	10	10 12	4 13
6	750	9 0	10	20.12	7 04
2	750	7 0	6	4.38	2 26
2	1000	5 0	7	3.82	2 09
4	1000	5 0	7	4 60	2.44
2	1000	7 0	10	4.60	2.46
4	1000	7 0	10	6 88	3 20
6	1000	7 0	10	10.64	4.26
8.5	1000	7 0	10	35 60	10.74
2	1000	7 4	15	5.60	2 60
4	1000	7 4	15	9.10	4 02
6	1000	7 4	15	17.85	6 32
2	2000	5 0	10	4.12	2.18
4	2000	5.0	10	5 70	2.80
6	2000	5.0	10	9.81	4.00

ated at reduced pressure (40 to 43 mm.). A slow stream of hydrogen was passed through the oil, causing the oil to froth and be well mixed during the discharge. Table 24 gives conditions studied and the viscosity of the products formed (249).

From the data the following conclusions were drawn: (1) With constant electrical conditions, the viscosity increases with time, at first slowly and later more rapidly, especially after 6 hr. (2) When the frequency and time are constant, the viscosity increases with the power in the discharge. (3) The optimum frequency is 500 cycles per second. This may be limited by the apparatus and kerosene used in the study. (4) To produce a given change in the properties of the oil under treatment, a *much* larger change must be made in the electrical conditions.

In conclusion it may be said that, so far as the writers know, these electrically treated oils are not being produced commercially in the United States. They are being produced in Belgium and Germany.

C. PRODUCTION OF MOTOR FUELS

Electrical methods of producing acetylene and lubricating oils depend upon the application of rather specific conditions to produce the desired results. By the application of still other conditions it is possible to take advantage of the electrical discharge to produce hydrocarbons boiling in the gasoline range and suitable for motor fuels.

The simplest process of this type consists in vaporizing or atomizing the oil to be cracked into a zone where a discharge is occurring and removing the reaction products after a given length of time (52, 66, 86, 95, 113, 129, 214, 226, 231, 255).

There are several variations on this general theme. The one most widely mentioned is the mixing of hydrogen, a hydrogen-containing gas, methane, coal gas, the gas produced in the process, or even water vapor with the hydrocarbons undergoing treatment. It is indicated that in this way the hydrogen can be transferred to quite heavy oils and tars with the production of gasoline (51, 95, 199, 220, 239, 256, 257). Catalysts may or may not be present.

The Cherry process utilizing this principle has been the subject of rather intensive development in the past but has not survived in competition with the thermal-pressure process of cracking oil. It is indicated that the methane or hydrocarbon gas acts as a carrier for the hydrocarbon vapors and furnishes hydrogen so that carbon formation is prevented in the reaction zone. The process resembles the "vapor-phase" process for cracking petroleum, using substantially atmospheric pressure. The hot oil vapors are passed through corona discharge chambers made of metal and arranged so that the inner electrode is insulated from the outer vessel. A fused-

quartz insulated lead-in wire connects with the inner electrode. The normal conversion to gasoline is 16 to 25 per cent upon passage through the process. If the process is run without energizing the corona tubes, it is found that the conversion to gasoline drops and the antiknock quality of the gasoline is lower.

The gasoline produced is highly olefinic and resembles "vapor-phase" gasoline in many of its characteristics, especially since it requires special methods of treating to make a marketable product. The gasoline is said to be high in antiknock quality. The reported range was 54 to 77 "benzol equivalent". From this we estimate 68 to 80 A.S.T.M. motor method (50, 219).

The next variation in the electrical process of making gasoline is to combine the use of thermal energy, catalysts, and electrical energy. The catalysts which have been proposed include carbon, uranium, radium, thorium, nickel, silver, cobalt, iron, magnesium, copper, molybdenum, lead, tantalum, and tungsten. The process may be varied so that the catalyst and the electrical energy act on the oil at different stages or they may be combined. One of the methods proposed for effecting this combination has been to maintain a high-frequency field in the catalyst bed (2, 76, 152, 238).

The high-frequency field has also been proposed without the use of any intentional catalyst (114).

It is indicated that natural gas, methane, or other hydrocarbon gases can be converted into gasoline by the simultaneous action of a high-frequency current and a catalyst consisting of coke impregnated with cuprous chloride (190).

Petroleum cracking is also accomplished by passing hydrocarbon gases through an arc, so that highly active particles are formed and pass directly from the arc into oil vapors preheated to 425°C. (244).

D. MISCELLANEOUS PROCESSES

It has been proposed to crack petroleum gases or coal gas in high-tension silent discharges to lower the molecular weight, decrease the condensing point, and increase the volume (61). Conversely, it has also been proposed that motor fuels and lubricants be made from methane in the silent discharges (74, 75, 211).

Silent discharges in hydrocarbon gases in the presence of metallic lead have been used to produce lead compounds for raising the antiknock quality of gasoline (246). The same use has been made of arc discharges (245).

It has also been conceived that a petroleum reaction chamber might be made like a giant radio tube having a heated filament to supply electrons by thermionic emission and a charged plate to accelerate the electrons

across the reaction zone. The accelerated electrons would cause chemical reaction (183).

The glow discharge in benzene is a potential method for making diphenyl (143).

The manufacture of "higher" olefins and acetylenes by treating ethylene in the arc (84), butadiene from fuel oil in the arc (59), and olefins and diolefins by passing hydrocarbon gases through granular materials heated by an arc (125) has been patented.

VI. GENERAL DISCUSSION

It is readily apparent from the preceding pages that electricity is a powerful tool for causing hydrocarbons to react. So powerful is this tool that the full technique for controlling it completely to cause only desired reactions is not yet known. Yet enough is known to show that many useful reactions can be effected in this manner and, as this control technique is learned, more of these reactions will be used on a commercial scale.

As the main reactions of the discharges are (1) dehydrogenation-hydrogenation and (2) polymerization (condensation)-depolymerization, probably the earliest advances in controlling the reaction will be to separate these reactions so that any one can be used to the exclusion of the other. As this is done the reactions may be used to make rubber, synthetic resins and plastics, drying oils, antiknock motor fuel, and tough lubricants. Most of the principles are known and most of the reactions are known; the optimum conditions remain to be found. There is every reason to believe that in most cases the power consumption will be within economic reason when these optimum conditions are found.

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THE NATURE OF ENERGETIC COUPLING IN BIOLOGICAL SYNTHESSES

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Received October 22, 1940

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I. INTRODUCTION

The purpose of this review is to acquaint chemists with the great advances made recently in that branch of biological chemistry which is concerned with the chemical mechanism of cellular respiration and the nature of energetic coupling.

Biologists have for a long time been aware that, in addition to simple physicochemical processes (filtration, diffusion, osmosis, ionic reactions, hydrolysis), living organisms depend on other processes of a more complicated nature. These, the vital processes, include motion, transmission of nerve impulses, secretion, growth, etc., which take place only if furnished with energy developed from the oxidation of foodstuffs (metabolites) either by oxygen (respiration) or by double bonds occurring in organic substances (dismutations and fermentations).

When a kidney is perfused with oxygenated blood according to the method of Starling (261), normal urine is produced as the result of a simple ultrafiltration of blood plasma and a selective reabsorption of the main part of the water and all the glucose from the ultrafiltrate. If, however, the respiration of the kidney tissue is stopped by the addition of cyanide, the volume of the urine increases enormously and sugar is excreted, since urine excretion under such conditions is merely the result of a simple ultrafiltration process (16).

The secretion by glands and the transmission of impulses in the nervous system are also processes which depend on cellular respiration.

The contraction of muscles is dependent on energy which, however, does not require respiration, since a frog muscle in an oxygen-free at-

mosphere is able to carry out several hundred single contractions or a tetanus of long duration. As shown by Meyerhof (198), the energy supply in such cases is derived from an internal oxidation of sugar: this process leads to the formation of lactic acid and is called glycolysis or animal fermentation.

Lundsgaard (185), in connection with his discovery of the complete inhibition of lactic acid formation by iodoacetic acid, observed that even when glycolysis is stopped a muscle is able to contract to a limited extent. After forty to fifty single contractions a muscle poisoned with iodoacetic acid is exhausted, the contractions decrease rapidly, and simultaneously the relaxation becomes more and more incomplete, i.e., the muscle is completely exhausted in a state of rigor. Lundsgaard further demonstrated that in muscles which contract without respiration or fermentation the liberation of inorganic phosphate from certain phosphate esters (creatine phosphate, adenosine polyphosphate) takes place to a much greater extent than in normal muscles. Apparently respiration and fermentation restore these phosphate esters, whereas muscles poisoned with iodoacetic acid and without oxygen supply very soon consume the limited stores of creatine phosphate and adenylic polyphosphates which appear to be the most direct energy source for the contraction mechanism. Thus Lundsgaard observed that, simultaneously with the appearance of rigor and complete exhaustion, the supply of creatine phosphate was used up and that of adenylic polyphosphate greatly diminished. These fundamental experiments therefore indicate that the "level" of creatine phosphate + adenosine polyphosphate is maintained by oxidoreductions (respiration or fermentation), whereas the contractile system is "charged" by energy supplied by the liberation of inorganic phosphate from creatine phosphate and adenosine polyphosphates. Since muscles, contracting at the expense of creatine phosphate and adenosine phosphates alone, are exhausted in a state of rigor, the energy furnished by these two dephosphorylations most likely is used not for the contraction but for the relaxation of the contracted muscle, and the relaxation therefore is probably the charged state of the contractile system (see section X).

The restoration of creatine phosphate and adenosine polyphosphates by oxidoreductions will be discussed in detail in this review, since the mechanism of this coupling is understood to a considerable extent. This understanding is mainly due to the magnificent work of Warburg and coworkers (292, 299, 220) who, by revealing the nature of oxidoreduction enzymes, have made it possible to understand chemically how the energy of respiration and fermentations can be utilized for biological syntheses. The phosphorylations of creatine, adenylic acid, or glucose offer a good illustration of biological syntheses which are coupled to oxidation.

The term "synthesis" as applied in organic chemistry merely means the conversion of one substance into another more complex substance, regardless of thermodynamic concept. In biology the term "synthesis or assimilation", however, has for quite a long time been applied to reactions which lead to an increased free energy (positive ΔF) of some of the members of a given system.

Since an increase of free energy² as an end result is thermodynamically impossible, biological syntheses must be characterized by a coupling between two reactions, the one representing an increase in free energy ($+\Delta F_1$), the other a fall ($-\Delta F_2$), where ΔF_2 has to be equal to or larger than ΔF_1 . Such a definition of biological syntheses includes, in addition to highly complex reactions, certain relatively simple processes, such as the dismutation of sugars into polyalcohols and sugar acids or alcoholic fermentation. Most biologists would hesitate to call alcoholic fermentation a synthesis, because of the liberation of energy ($-\Delta F_2$ much greater than $+\Delta F_1$) and the formation of substances of lower molecular weights than the original.

Nevertheless it is important to realize that if the conversion of sugars into fatty acids is called a synthesis (assimilation), and it is generally so termed among biologists, several dismutations and fermentations also belong to the group of syntheses, owing to the similarity of these last processes to the conversion of sugar into fatty acids (see section III).

Since the formation of polysaccharides from monosaccharides requires a supply of energy, ordinarily derived from biological oxidations, this process is generally considered as a typical synthesis. Frequently, however, emphasis has been placed on the phenomena of polymerization. In this connection it is important to appreciate that the enzymatic dimerization of triose phosphate into hexose diphosphate (202, 203) and the polymerization of glucose-1-phosphate into polyhexoses (45, 130) have been shown to proceed directly, resulting in a small decrease in free energy. Calling such polymerizations syntheses would imply that the concept of biological syntheses involves no consideration of thermodynamics whatsoever.³

Some biologists, regarding the increase of free energy as being an essential factor in syntheses, furthermore require a coupling between qualitatively different systems like the just-mentioned uptake of inorganic phosphate into organic ester linkages. Here we have a coupling between two qualitatively different kinds of processes,—oxidation-reduction and phosphorylation.

² Concentration differences have also to be included in thermodynamic effects.

³ The dimerization of free radicals liberates a large amount of free energy ($-\Delta F$ is large).

Since the concept of synthesis seems to be of a very subjective nature, I think that it should be abandoned in purely thermodynamic considerations.

Thermodynamically a process can be described as having a negative or a positive change in free energy ($-\Delta F$ and $+\Delta F$). If ΔF is negative, the reaction can occur and is able to produce work; if ΔF is positive, work must be expended to cause the reaction to occur. The first kind of reaction has been termed "exothermic", the last kind "endothermic". Recently Coryell (55) has recommended that terms like "exothermic" and "endothermic" be applied only to characterize negative and positive changes in heat ($\pm\Delta H$), and that the new terms "exergonic" and "endergonic" be applied to characterize changes in free energy (ΔF). "Ergonic" is derived from the Greek *ergon*, meaning work. Coryell's terminology will be used in the present review.

	-	+
ΔH	Exothermic	Endothermic
ΔF	Exergonic	Endergonic

In biology the concepts of assimilation (or synthesis) and dissimilation still keep their importance. A distinction between fermentations acting as purely dissimilatory systems and those acting as assimilatory as well as dissimilatory systems is very useful. The conversions of sugar into lactic acid and of glycerol into propionic acid (227) offer examples of purely dissimilatory fermentations, i.e., fermentations where the end product has lower energy content than the starting substance. The conversion of sugars into carbon dioxide and ethyl alcohol or into carbon dioxide, acetic acid, and propionic acid or butyric acid offer good illustrations of fermentations which act as assimilatory as well as dissimilatory systems. One part of the sugar molecule is sacrificed as carbon dioxide in a reaction which is "coupled" with the endergonic ($+\Delta F$) conversion of the other part into ethyl alcohol or fatty acids. The main part of this review will deal with the chemical nature of the coupling between exergonic ($-\Delta F$) and endergonic ($+\Delta F$) processes, starting with couplings between oxidoreductions and reactions of different kinds, particularly phosphate esterifications.

In an interesting review by A. J. Kluyver in 1931 (133), describing the state of understanding of biological assimilations and dissimilations at that time, he expresses the value of a coördination of experimental facts, as follows: "Personally I have no hesitation in asserting that even for an experimental science such as biochemistry the day will come when it will be wise to pause for a short time and say 'Enough matter, more art'. And

when we consider for a moment the mass of facts that has already been gathered in the biochemical field, it seems to me that this day of retrospection and synthesis should not be far off".

During the last five years a large amount of information has been collected regarding the details of biological couplings which justifies a survey of the principles which underlie biological syntheses.

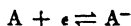
II. THE PRINCIPLES OF OXIDATION-REDUCTION

A. The characteristics of oxidoreductions

It seems advisable, before continuing the consideration of fermentations and assimilations, to give a very condensed summary of some characteristics of oxidation-reduction which are important in connection with the problems to be discussed. This section will deal only with the elementary oxidoreduction process; the specific enzymes which catalyze oxidoreduction systems will be described in a later section.

W. M. Clark (37) was the first to realize that electron transfer was the common feature of all biological oxidoreductions. When presenting a condensed summary of this subject, a quotation from a review by Michaelis and Schubert (210) is very useful:

If a substance, A, can undergo a reversible reduction by accepting an electron (e) the process may be represented thus:



If the product, A^- , happens to be the ion of a weak acid it will tend to combine with a proton furnished either by the oxonium ion OH_4^+ , if the solvent contains water, or by any other acid in Bronsted's generalized terminology:



If these two processes occur simultaneously the net effect can be represented by the combined reaction:

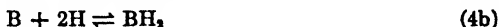


or

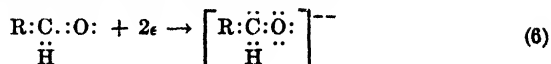


Only in such a case is the term "reduction" entirely equivalent to hydrogenation.

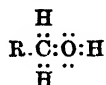
Exactly the same argument applies to the bivalent oxidation-reduction process of a substance B when the two steps occur simultaneously. The corresponding reactions are:



The electronic structures corresponding to equation 4 are:

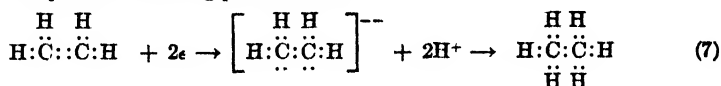


The product formed would be the divalent ion of an unmeasurably weak acid and would immediately attach protons to form the alcohol:



But this addition of protons has nothing to do with the reduction process, which is entirely contained in equation 6.

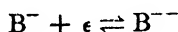
A similar case is afforded by the reduction of ethylene to ethane, in which the reduction and proton-attaching processes are:



The pairs of molecules AH and A^- or BH_2 and B^{--} of equations 2 and 4 may be said to be two states of ionization of the same acid, of which the ionized form in some cases may be practically incapable of existence.

Semiquinones

In a certain number of instances it has been demonstrated that the bivalent redox process illustrated in equation 2 actually occurs in two successive univalent steps involving a half-reduced (or half-oxidized) intermediate.



This intermediate, which contains an unpaired electron and therefore has the character of an organic free radical, is called a semiquinone (208). The investigations of Shaffer in 1933 (257) showed the importance of one-step oxidation for inorganic redox systems, and a few years later Michaelis and collaborators (208) showed the occurrence of one-step oxidation in a number of important organic redox systems. Michaelis and collaborators were able to demonstrate the occurrence of semiquinones electrometrically and by the magnetic susceptibility due to the unneutralized spin of the odd electron (210, page 441):

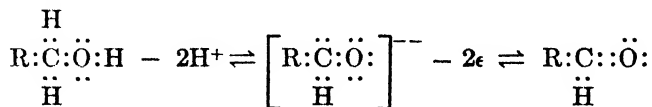
It is an essential property of these intermediate oxidation levels that they are always in a mobile equilibrium with the compounds on the next higher and the next lower step of oxidation, whereas ordinary valence-saturated organic compounds are usually inert with respect to establishing equilibria with other valence-saturated compounds. Acetaldehyde does not dismute to ethyl alcohol and acetic acid, . . . although it would be possible, speaking purely thermodynamically. In contrast, the establishment of the equilibrium of a radical of the type mentioned with an

electron donor or acceptor is just as unhampered as that of an acid or base with a proton donor or acceptor.

In a later section in this review the one-step oxidation will be discussed again.

B. Redox potentials and chemical structure

As a consequence of the formulation of equations 1 or 6 and 7 it follows that in oxidizing a substance AH_2 a removal of protons (proton dissociation) would have to precede the removal of electrons:



The ionization of the electron donor may be a factor of importance for the emission of electrons and may account for the fact that potentials of biological oxidoreduction systems as a rule drop rapidly (i.e., increasing ability to emit e) in going from systems where the electron donor is an immeasurably weak acid to systems where the electron donor has a measurable H^+ dissociation.

In table 1 the redox potentials of some typical metabolites are presented, using the system of W. M. Clark.

Positive redox potentials mean oxidizing systems; negative ones mean reducing systems. A positive ΔF means that a spontaneous reaction is impossible; ΔF negative means that a spontaneous reaction is possible.

$\Delta \bar{F}$ represents the free-energy change (in calories) under standard conditions, i.e., 1 molar (unit activity) water solution, pH 0, temperature 25°C . ($T = 298^\circ$). $\Delta \bar{F} = nF\bar{E}$, where \bar{E} designates the normal potential (red./ox. = 1) under standard conditions, n is the number of electrons involved in the redox process, and F is the Faraday constant. E'_0 represents the normal potential under conditions other than standard, i.e., when the activity of the reactants is not unity and the pH is different from 0.

Many of the redox potentials have been obtained only from thermal data. Owing to the fundamental work of Parks and Huffman (237) and of Huffman and Borsook (116) and to later work (cf. 24), very exact data for the free energy of formation of a number of important biological substances have been obtained.⁴ This has made it possible to obtain redox potentials independently of direct potentiometric measurements.

⁴ Franke in 1933 pointed out in an interesting review (86) the essential importance of thermal data for the understanding of metabolic processes. Franke's calculations were, however, very rough and therefore not able to demonstrate the exact agreement between thermal data and potentiometric measurements.

There is excellent agreement between the accurate potentiometric measurements of Lehmann (161) and of Borsook and Schott (27) and the redox potentials calculated from thermal data (Parks and Huffman). Very good agreement also has been obtained for other redox systems. Owing to the new methods of specific heat determination developed by Rossini, Huffman, Parks, and others, very exact values for entropies will be possible and therefore also more accurate values for free energies and free-energy changes. Furthermore, W. M. Clark (35) and, more recently, Borsook (26) have calculated redox potentials from equilibrium constants and have thus obtained relatively accurate data for some systems where thermal data have not yet been obtained.

It is well known that the redox potential varies with pH according to the equation:

$$E'_0 = \tilde{E} - N \frac{RT}{n_2 f} \times \text{pH}$$

if $n/N = 2$,

$$E'_0 = \tilde{E} - 0.03 \times \text{pH}$$

If $n/N = 1$,

$$E'_0 = \tilde{E} - 0.06 \times \text{pH}$$

n designates the number of electrons and N the number of protons involved in the redox process. In the oxidation of carbonyl to carboxyl, $n/N = 2/3$.

Most biological hydrogen donors are oxidized in two steps according to the reaction:



and the pH curve (at 30°C.) therefore follows the 0.06 slope. If in a two-step oxidation (removal of $2e$) only one proton is removed from the group which is involved in the oxidation, the pH curve will follow the 0.03 slope. The cozymase (pyridine nucleotide) represents a system which has a 0.03 slope of the redox pH curve. The 0.03 pH curve shows that only one proton is removed when two electrons are removed, in agreement with the nature of the group which is oxidized or reduced (see section IV).

According to the equation

$$E'_0 = \tilde{E} - 0.0615 \times \text{pH}$$

the normal potential of a redox system in which $2e$ and 2H^+ are involved increases 430 millivolts (= 19.096 calories) when the pH is changed from 7 to 0. A corresponding change in pH will, however, increase the redox potential of the pyridine system only 215 millivolts (*cf.* Borsook (25, 26)).

TABLE I
Redox potentials of some typical metabolites

KIND OF REDOX SYSTEM	METABOLITES*	$\Delta F^\circ = nF\Delta E^\circ$ i.e., at unit activity, pH 0, $T = 25^\circ$ calories	E° (pH 7) milli- volts	REMARKS
$\begin{array}{c} \text{H} & \text{H} & & \\ & & & \\ -\text{C}-\text{C}- & \rightleftharpoons & -\text{C}=\text{C}- & + 2\text{H}^+ + 2e \\ & & & \\ \text{H} & \text{H} & \text{H} & \text{H} \end{array}$ <p>Paraffins to olefins</p>	I			
	(1) Succinate $^{2-} \rightleftharpoons$ fumarate $^{2-} + 2\text{H}^+ + 2e$	+20 450	± 0	Full agreement between potentiometric measurements (161) and thermal data (237)
	(2) Propionate $^- \rightleftharpoons$ acrylate $^- + 2\text{H}^+ + 2e$	+20 660	+10	ΔF° calculated from ΔH , ΔS estimated from analogous cases (Huffman). E° calculated from ΔF° (Borsook (26))
	(3) Butyrate $^- \rightleftharpoons$ crotonate $^- + 2\text{H}^+ + 2e$	+19 066	-25	
	(4) Palmitate $^- \rightleftharpoons$ oleinate $^- + 2\text{H}^+ + 2e$	$\Delta F^\circ =$ +21 440	+25	Since these fatty acids are water-insoluble, only ΔF° can be calculated
$\begin{array}{c} \text{H} \\ \\ -\text{C}-\text{COO}^- + \text{H}_2\text{O} \rightleftharpoons -\text{C}-\text{COO}^- + \text{NH}_4^+ \\ \\ ^+\text{NH}_2 \end{array}$ $+ 2\text{H}^+ + 2e$ $\left(\begin{array}{c} \text{H} \\ \\ \text{C} \\ \\ \text{NH} \end{array} \right)$ <p>Passing the $-\text{C}-$ step</p> <p>i.e., amino \rightarrow iminocarbonyl</p>	II			
	(1) Alanine $^- + \text{H}_2\text{O} \rightleftharpoons$ pyruvate $^- + (\text{NH}_4)^+ + 2\text{H}^+ + 2e$	+18 380	-40	E° measured by Wurmser and Wurmser (310); ΔF° calculated from E° (26)
	(2) Glutamate $^{2-} + \text{H}_2\text{O} \rightleftharpoons$ α -ketoglutarate $^{2-} + (\text{NH}_4)^+ + 2\text{H}^+ + 2e$	+18 820	-30	ΔF° and E° calculated from II(1) and from the transamination equilibrium (see Borsook (26))

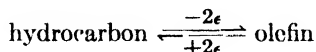
<p>III</p> $\begin{array}{c} \text{H} \\ \\ \text{---C---} \rightleftharpoons \text{C---} + 2\text{H}^+ + 2e \\ \\ \text{OH} \\ \text{ } \\ \text{O} \end{array}$ <p>Hydroxy (alcohol) to carbonyl</p>	<p>(1) Malate²⁻ \rightleftharpoons oxaloacetate²⁻ + 2H⁺ + 2e (2) Lactate⁻ \rightleftharpoons pyruvate⁻ + 2H⁺ + 2e (3) Ethyl alcohol \rightleftharpoons acetaldehyde + 2H⁺ + 2e (4) β-Hydroxybutyrate⁻ \rightleftharpoons acetoacetate⁻ + 2H⁺ + 2e</p>	<p>+15 500 +11 880 +11 730 +6 580</p>	<p>-102 <i>E'</i> measured by Lehmann and Jørgensen (162) -180 <i>E'</i> measured by Barron and Hastings (15) -163 ΔF° and <i>E'</i> calculated from Negelein and Wulff and from the potential of coenzyme (Borsock (26)) -290 <i>E'</i> measured by Jørgensen (115)</p>
<p>$\text{H}_2 \rightleftharpoons 2\text{H}^+ + 2e$ (hydrogen electrode)</p> $\begin{array}{c} \text{H} \quad \text{O}^- \\ \diagdown \quad \diagup \\ \text{C}=\text{O} \end{array} + \text{H}_2\text{O} \rightleftharpoons \begin{array}{c} \text{H} \quad \text{O}^- \\ \diagdown \quad \diagup \\ \text{C}=\text{O} \end{array} + 3\text{H}^+ + 2e$ $\begin{array}{c} \text{H} \quad \text{OH} \\ \diagdown \quad \diagup \\ \text{C}=\text{O} \end{array} + \text{H}_2\text{O} \rightleftharpoons \begin{array}{c} \text{H} \quad \text{OH} \\ \diagdown \quad \diagup \\ \text{C}=\text{O} \end{array} + 2\text{H}^+ + 2e$	<p>IV</p> <p>(1) Acetaldehyde (+H₂O) \rightleftharpoons acetate⁻ + 3H⁺ + 2e (2) Sugar (+H₂O) \rightleftharpoons sugar acid⁻ + 3H⁺ + 2e (3) Hypoxanthine + H₂O \rightleftharpoons xanthine + 2H⁺ + 2e (4) Xanthine + H₂O \rightleftharpoons uric acid + 2H⁺ + 2e</p>	<p>0 -1 790</p>	<p>-430 -468 ΔF° calculated from Parks and Huffman (237) -400 <i>Cf.</i> Green <i>et al.</i> (99). The biological sugar oxidations seem to involve phosphate instead of water (Negelein and Brömel (220)) -407 Potentiometric measurements with methylviologen (Filitti (81)) -355 Potentiometric measurements (Green (95))</p>
<p>V</p> $\begin{array}{c} \text{O}^- \\ \diagdown \quad \diagup \\ \text{C}=\text{O} \end{array} + \text{H}_2\text{O} \rightleftharpoons \begin{array}{c} \text{O}^- \\ \diagdown \quad \diagup \\ \text{C}=\text{O} \end{array} + \text{H}_2\text{(gas)}$ $\begin{array}{c} \text{OH} \quad \text{OH} \\ \diagdown \quad \diagup \quad \diagdown \quad \diagup \\ \text{C}=\text{O} \quad \text{C}=\text{O} \end{array} + \text{H}_2\text{O} \rightleftharpoons \begin{array}{c} \text{OH} \quad \text{OH} \\ \diagdown \quad \diagup \quad \diagdown \quad \diagup \\ \text{C}=\text{O} \quad \text{C}=\text{O} \end{array} + \text{CO}_2 + 2\text{H}^+ + 2e$	<p>(1) Formate⁻ \rightleftharpoons CO₂ + H₂(gas) (2) Pyruvate⁻ + 2H₂O \rightleftharpoons acetate⁻ + HCO₃⁻ + 3H⁺ + 2e (3) α-Ketoglutarate²⁻ + 2H₂O \rightleftharpoons succinate²⁻ + HCO₃⁻ + 3H⁺ + 2e</p>	<p>-425 <i>E'</i> measured as an equilibrium constant by Woods (309). ΔF° calculated from Parks and Huffman. The gas pressure replaces the hydrogen-ion concentration -630 ΔF° calculated from thermal data and from II(1) and II(2) (Borsock (26)). The biological oxidation of pyruvic acid involves phosphate instead of water (Lipmann (175)) -8.000 ΔF° calculated from thermal data and from II(1) and II(2) (26)</p>	

* The metabolites are not ionized at standard conditions.

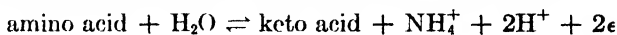
† ΔF° is the change in free energy between pure solids or liquids.

It is obvious, therefore, that the relationship between the redox potentials of various systems depends upon the pH at which they are compared. Since n/N varies from 0.67 to 2, the comparison between redox potentials of biologically important substances must be made at a pH near that of the tissue, for instance, at pH 7.

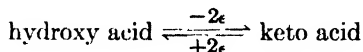
Table 1 shows that the system



is the most positive system of all metabolites. The system



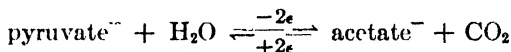
forming the imino acid as the primary product, is considerably more positive than the system



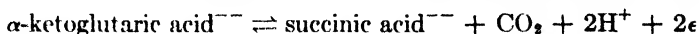
This fact might explain the observation of Krebs and Cohen (150) that the dismutation of α -ketoglutaric acid is increased markedly in the presence of ammonia

The potential of the sugar-sugar acid system is not known exactly. It seems, however, quite certain that phosphorylation of aldehyde and carboxyl groups raises the potential considerably (see section V).

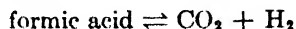
Bor-ook's calculations (26) of the free-energy change in the system



shows a very strong negativity of this system, approximately 200 millivolts more negative than the hydrogen electrode. The enzymatic system has never been measured potentiometrically. The recent discovery of Lapmann (174) that phosphate is involved in the enzymatic oxidation of pyruvic acid will be discussed later; in the same place it will also be understood why the enzymatic redox system must have a normal potential considerably more positive than that calculated from thermal data. This fact does not diminish the great biological importance of the redox potential calculated from thermal data, since the end result of the enzymatic oxidation of pyruvic acid is acetic acid and carbon dioxide and the phosphate does not enter the final balance. It is also interesting that the system



which is so closely related to the pyruvic acid-acetic acid system, also has a redox potential more than 100 millivolts more negative than the hydrogen electrode (26). The system



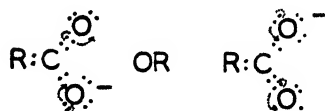
is very near the level of the hydrogen electrode (309).

If hydrogen gas (H_2) is formed, the hydrogen pressure rather than the hydrogen-ion concentration determines the redox potential. Woods' equilibrium determinations (309) were carried out at an alkaline reaction (pH about 8) but at a hydrogen pressure very near 1 atmosphere, i.e., under conditions corresponding to pH 0 for redox systems which do not form hydrogen gas.

Looking at table 1, we find as a general feature that the higher the proton dissociation (acidic properties) the better the electron donor, i.e., reducing agent. The degree of proton dissociation of the group to be oxidized is not, however, the general factor which determines the potential of redox systems.

The high potentials of systems like dienols \rightleftharpoons diketones, diphenols \rightleftharpoons quinones, nitrite \rightleftharpoons nitrate, sulfite \rightleftharpoons sulfate contradict the proton hypothesis just mentioned. One factor, however, seems to be able to account for all the facts concerning oxidoreduction potentials,—*viz.*, the so-called resonance energy. It would be out of place here to attempt even an outline of this interesting development in modern physical chemistry. This field has been described in the fundamental monograph of G. N. Lewis (166) and in the comprehensive monograph of L. Pauling (240). A study of these two monographs, supplemented by discussions with Dr. Coryell, has led the author of this review to believe that the modern concepts of structural chemistry will actually be able to account for the thermodynamic properties of biological redox systems in general and phosphoric esters in particular.

In trying to illustrate the great importance of resonance for biological oxidoreduction, let us examine, for instance, a carboxyl group, written in the electronic terminology introduced by G. N. Lewis in 1916 (*cf.* 166). A carboxyl group, which is ionized, can be illustrated by two equivalent structures:



The calculation of the stability of the carboxyl group from the sums of double-bond and single-bond energies gives a much lower value than that found experimentally. This extra stability is called resonance energy (240). Owing to the resonance energy, such structures as the carboxylate ion (with resonating structures) are characterized by a high degree of stability; this means that a large amount of energy is necessary to transform such structures into other groups not belonging to the resonating type, as is the case, for instance, with aldehydes. The fact that carboxyl groups have a very small tendency to accept electrons, i.e., are poor oxidiz-

ing agents, may be attributed to the high resonance energy they have in relation to the hydrogenated product, aldehydes. Since carbon dioxide has more resonance than the carboxylate ion, this last group of compounds in the table are very good electron donors. The ethylene group, having no resonating structure, is known to have a large tendency to accept electrons. Quinones have less resonance than hydroquinones, a fact which agrees with the strong oxidizing action of the diketo groups. Nitrates and sulfates, having high resonance energies, can be reduced to nitrites and sulfites, which also possess a high degree of resonance; the small change in stability means that nitrates and sulfates are good electron-acceptor systems. The much larger stability of water than that of oxygen, due to the ionic character of O—H bonds in water, lends the latter substance a large affinity for electrons.

In the section dealing with the coupling between oxidoreduction and phosphorylations I shall return to the field of structural chemistry which is of direct significance for this problem.

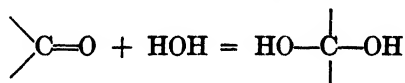
Regarding the concepts of oxidoreduction potentials and free-energy changes, it is always important to bear in mind that what determines whether an oxidation or a reduction of a substance is endergonic ($+\Delta F$) or exergonic ($-\Delta F$) is the change in the stability of the molecular group involved in such reactions. Going from more stable to less stable structures means an increase in free energy ($+\Delta F$) and *vice versa*. In order to know the stability of structures, the conditions under which the change is assumed to occur have to be stated, whether at unit activity and pH 0, usually called standard conditions, (\bar{F} , \bar{E}), or at pH 7, etc. I emphasize this, because in a recent review Kollath and Stadler (142) define a reduction as "Energiebindung" and an oxidation as "Energieabgabe."⁵ Such a definition is not only misleading but wrong. Quite apart from the fact that the authors in their definition do not include the concentration of the substances involved in oxidoreductions, particularly the hydrogen ions, one of the consequences of their statement is that the reduction of oxygen, known to be an extremely exergonic reaction (high negative ΔF), is an "Energiebindung." The transfer of electrons from one iron porphyrin system to another having a more "positive" redox potential (i.e., a stronger oxidizing system) is known to be a spontaneous reaction, which therefore represents an exergonic process. Since the E'_0 values (pH 7) of iron porphyrins are positive (i.e., the removal of electrons from such compounds represents an endergonic process), the release of energy in transferring electrons from one "positive" system to another more "positive" must be due

⁵ "Unter Reduktion versteht man chemische Vorgänge in der Materie, die mit Energiebindung, unter Oxydation solche, die mit Energieabgabe einhergehen." (142)

to the reduction of the most "positive" system. These examples are sufficient to illustrate that the definition proposed by Kollath and Stadler does not make any sense whatsoever.

C. Hydrogen donors and acceptors

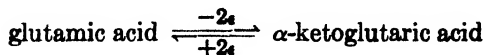
Since protons as well as electrons are involved in most biological oxidoreductions, it has been customary to identify oxidation with dehydrogenation and reduction with hydrogenation. On several occasions it is therefore convenient to use the Wieland-Thunberg terminology: "hydrogen donors" for the substances which emit protons and electrons, and "hydrogen acceptors" for substances which take up protons and electrons. Wieland (302) was the first to advance the idea that carbonyl groups in order to be oxidized have to form hydrates:



This idea was partly based on model experiments which showed that a number of aldehydes in order to be oxidized require water. Aldehydes which form stable hydrates (e.g., mesoxalic acid) are oxidized in media free from water.

Hydrogen (or electron) acceptors have structures which possess double bonds. In several cases such double bonds are the result of anhydride formation as, for instance, in unsaturated fatty acids ($-\text{C}=\text{C}-$) which are the anhydrides of hydroxy acids, or in substances which can be considered as anhydrides; for instance, the ketones of keto acids ($\begin{array}{c} | \\ \text{C=O} \end{array}$) are the anhydrides of carbonyl hydrates.

As a rule, the hydrogen acceptor of an oxidoreduction system has to belong to the same or a more positive redox system than the hydrogen donor. Hydrogen-transfer systems which transfer hydrogen from the hydrogen donor to the acceptor usually have normal potentials between those of the hydrogen donor and of the hydrogen acceptor. Exceptions, however, are known. The normal potential of the transfer system triphosphopyridine nucleotide is considerably lower than that of the system



nevertheless, this transfer system can accept the hydrogen of glutamic acid and transfer it to a more positive system. The reason for this surprising fact is that in the presence of oxygen practically all of the pyridine nucleo-

tide is kept in the oxidized form; in the absence of oxygen the dehydrogenation of glutamic acid by pyridine stops immediately.

This example illustrates that it is essential that the potential of the final hydrogen-acceptor system be higher than that of the hydrogen-donor system, or the oxidation of the hydrogen donor will stop very soon.

D. Internal and external oxidoreductions

The distinction between internal and external oxidoreductions depends on the manner in which the hydrogen acceptor is supplied, that is, whether it is formed from the hydrogen donor or whether it is supplied from an external source.

Internal oxidoreductions form the hydrogen acceptor from the hydrogen donor. Two main types exist: (1) Dismutations, where the hydrogen acceptor is formed by removal of water from the hydrogen donor or by the formation of a ketone from an aldehyde. The double bond thus created is the hydrogen acceptor proper. (2) Fermentations, where the hydrogen acceptor is formed by the removal of water from the first or second oxidation level of the hydrogen donor. The double bond thus created is the hydrogen acceptor proper.

External oxidoreductions do not use hydrogen acceptors formed from the hydrogen donor but take up hydrogen acceptors from the environment. If oxygen (O_2) is used, the process is called respiration. If nitrate (HNO_3) or nitrite (HNO_2) is used, the process is called denitrification. Analogous to this reduction is the reduction of sulfates and the reduction of carbonates (chemosynthesis). The uptake of nitrogen is, however, a different process because of the high stability of the $N \equiv N$ bond (166) (see section VII).

This review will deal mainly with the internal oxidoreductions, because of the close relation of these processes to biological synthesis. The only group of external oxidoreductions which will be examined here is respiration, since it is so closely connected with fermentations and synthesis.

E. Dismutations

A dismutation is an oxidoreduction involving a molecular group and the anhydride of this group, the first acting as an electron and proton donor, the latter as an electron and proton acceptor.⁶ The classical example is the dismutation of the malic acid-fumaric acid system into 50 per cent succinic acid and 50 per cent oxaloacetic acid. In the presence of an enzyme, fumarase, which occurs in all biological systems, malic acid is partly transformed into its anhydride, fumaric acid, and *vice versa*.

⁶ The conversion of methylglyoxal into lactic acid (226) represents an oxidation-reduction in the same molecule, the hydrated aldehyde group is the electron donor and the keto group is the electron acceptor.

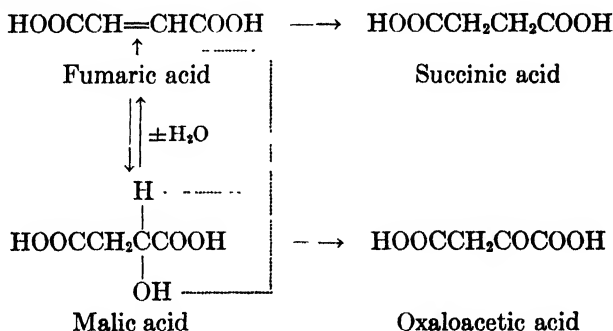
In potentiometric experiments with the succinic acid-fumaric acid system in the presence of the specific enzyme (but in the absence of fumarase), Lehmann (161) established that fumaric acid is reduced to succinic acid. The fine experiments of Lehmann furthermore illustrate the complete reversibility of the system



Moreover, experiments by F. G. Fischer (82) show the presence of an enzyme different from Lehmann's, capable of reducing fumaric acid to succinic acid, using a reduced dye (leuco-janus red) as hydrogen donor. The enzyme described by Fischer was called fumarate hydratase.

That malic acid, the hydrate of fumaric acid, is oxidized to oxaloacetic acid appears from experiments by Green (96) who, by preparing muscle extracts poor in fumarase, showed that the oxygen consumption starts immediately when malic acid is added, but only after an induction period when fumaric acid is added. This induction period is due to the slow conversion of fumaric acid into malic acid in such extracts.

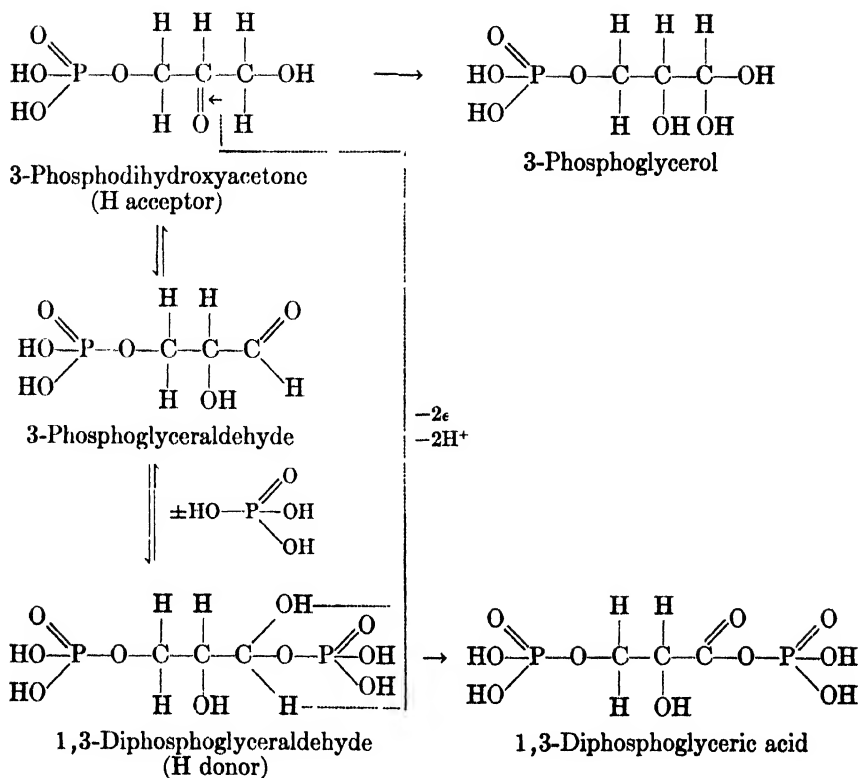
The dismutation of malic (fumaric) acid can be illustrated by the following scheme:



The dismutation of triose into glycerol and glyceric acid is a somewhat more complicated kind of dismutation, which requires phosphate (see page 88).

In the dismutation of triose into glycerol and glyceric acid, the ketotriose (dihydroxyacetone phosphate) acts as hydrogen acceptor, and the phosphorylated aldotriose (phosphate replacing water) as hydrogen donor. This has been established by important experiments of H. O. L. Fischer and Baer (84), of Kiessling and Schuster (131), and of Negelein and Brömel (220).

The dismutation of the fumaric acid-malic acid system is a confirmation of Wieland's assumption that hydrate formation creates hydrogen donors. The dismutation of triose phosphate and of pyruvic acid, however, shows, as pointed out by Lipmann (175), that phosphate in some cases replaces



water. Perhaps the significance of phosphate for biological dehydrogenations is greater than that of hydrate formation (*cf.* section V).

III. FERMENTATIONS

A. The characteristics of fermentations

The principles of fermentation are best illustrated in the simple case of lactic acid fermentation ("glycolysis"). The sugar is oxidized one level⁷ by an α -keto acid (pyruvic acid) which is thereby reduced to the hydroxy acid; the first oxidation product of the sugar (a sugar acid) is transformed into a keto acid by formation of the anhydride (*cf.* malic acid \rightleftharpoons fumaric acid). The hydrogen acceptor thus regenerated is able to oxidize a new molecule of the "active" sugar. Whereas a dismutation is oxidation of a compound by its anhydride (or keto-form), a fermentation in general can be characterized as an oxidation of a substance by the

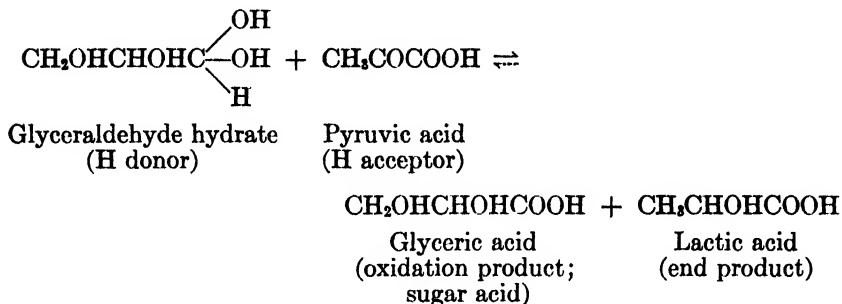
⁷ The expression "one-level" oxidation will be used to designate the oxidation of one valence-saturated state to the next, i.e., in most cases the removal of two electrons.

anhydride (or keto-form) of its own oxidation product, oxidized one or two levels higher.

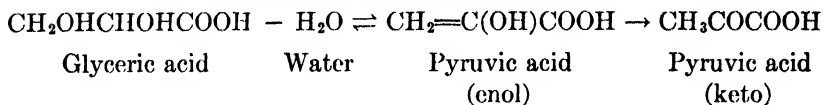
Glycolysis takes place on a large scale in animal tissue unable to use oxygen because of lack of oxygen or of oxygen-"activating" enzymes. In addition, the so-called lactic acid bacteria and some colorless algae (*Prototheca*) show this sort of fermentation in the absence of oxygen.

Disregarding the essential rôle of phosphate in oxidations at this point, the main features of the oxidoreduction of glycolysis can be illustrated as follows:

Oxidoreduction



Regeneration of the hydrogen acceptor



It is worth while to emphasize two characteristic features of glycolysis: (1) Sugar is converted quantitatively into one single substance, lactic acid, which has a lower energy content than the original. (2) No carbon dioxide is formed.

All the other sugar fermentations can be described as very simple variations of glycolysis, the variations occurring in most cases after the formation of pyruvic acid.

The pyruvic acid may undergo cleavage as follows: (a) Decarboxylation to acetaldehyde and carbon dioxide (alcoholic fermentation); (b) hydrolysis to formic and acetic acids (*coli* fermentation).

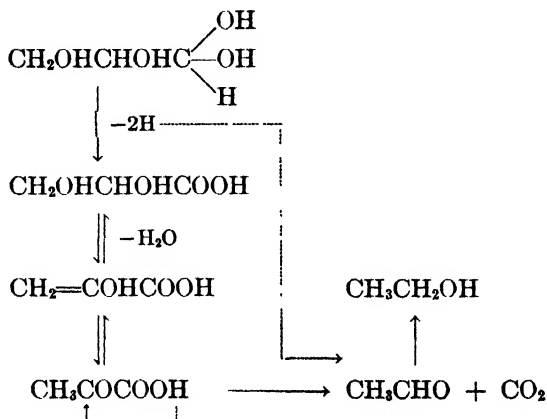
The pyruvic acid may be oxidized to acetic acid and carbon dioxide (fermentation of fatty acids).

The pyruvic acid may undergo condensations as follows: (a) with carbon dioxide to oxaloacetic acid (?) (*cf.* Wood and Werkmann (306) and Elsden (64)); (b) with amino acids as an acetylation (du Vigneaud (284)).

Other variations are due to secondary reactions of acetaldehyde, e.g.,

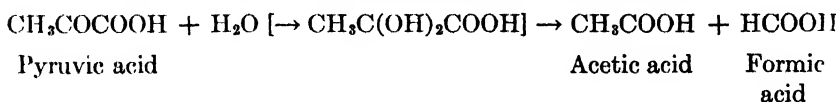
carbinol formation (*aerogenes* fermentation), or to secondary reactions of acetic acid, e.g., condensation to acetoacetic acid (*Clostridium butyricum*). These secondary products, replacing pyruvic acid as hydrogen acceptor, give rise to new end products.

The characteristics of *alcoholic fermentation* may be illustrated in the following way:

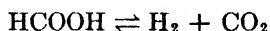


The scheme illustrates that acetaldehyde, not pyruvic acid, is the hydrogen acceptor in alcoholic fermentation. One of the end products, alcohol, has a higher energy content (per carbon atom) than sugar; the other end product, carbon dioxide, has a lower energy content. Whereas glycolysis is only a dissimilation, alcoholic fermentation is a mixture of dissimilation and assimilation.

In *coli* fermentation pyruvic acid is split in another manner:



The formic acid is degraded (decarboxylated or dehydrogenated) by a special enzyme into carbon dioxide and hydrogen:



Woods (309) has demonstrated the reversibility of the last reaction. Carbon dioxide can be reduced to formic acid not only by molecular hydrogen but also by organic hydrogen donors, a reaction described by Winogradsky (305) as early as 1890 (so-called chemosynthesis). It is of interest to investigate whether the reaction pyruvic acid = acetic acid + formic acid is reversible. A demonstration of the formation of pyruvic acid by the condensation of formic acid with acetic acid would make pos-

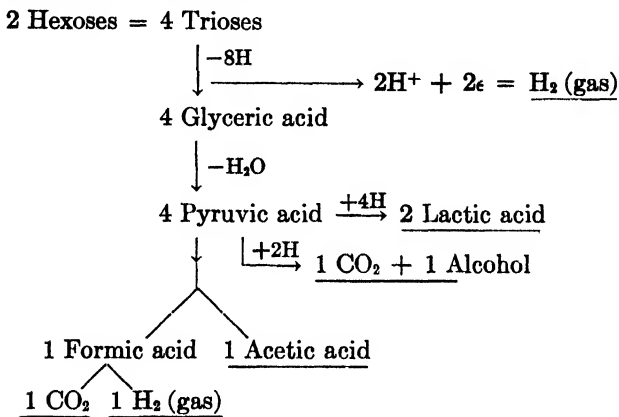
sible a complete chemical explanation of sugar formation from carbon dioxide.

We can summarize the facts known about *coli* fermentation as follows:

The equation of the ordinary *coli* fermentation (108) is



This equation is most readily explained by the following scheme:



Of the eight hydrogen atoms liberated from sugar, only six hydrogen atoms are used in the reduction of pyruvic acid and of acetaldehyde. The two hydrogen atoms left form 1 molecule of hydrogen gas.

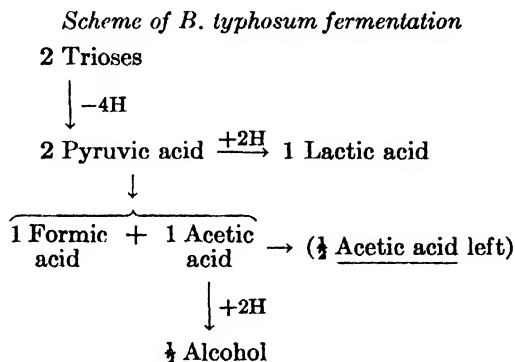
Adding the end products from the different processes we get: 2 lactic acid + 1 acetic acid + 1 alcohol + 2CO₂ + 2H₂.

Since 1 mole of alcohol is formed, 1 mole of carbon dioxide is derived from a simple decarboxylation of pyruvic acid which means, the ratio CO₂:H₂ being 1, that 1 mole of hydrogen is formed from the dehydrogenation of triose; this oxidation will just furnish the two hydrogen atoms left in the hydrogen balance. A consequence of these considerations is therefore that the electrode potential of the system triose-glyceric acid is not too far from the level of the hydrogen electrode.⁸ Stephenson and Stickland (263) have already pointed out that hydrogen must be formed from other sources than formate and have given evidence for this claim.

One objection against the scheme just presented is the fact that *B. typhosum* accumulates formic acid and does not form hydrogen (254). This might indicate that the only source of hydrogen in *coli* fermentation

⁸ This assumption is made on the basis of the old Wieland scheme of aldehyde oxidation. If phosphate is taken up in the aldehyde group (Negelein-Lipmann reaction) the oxidation can hardly yield hydrogen gas.

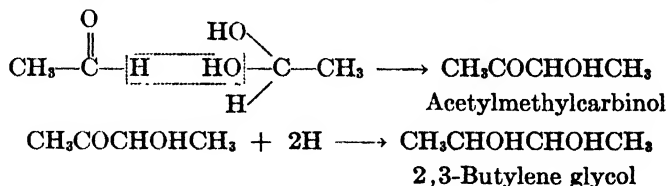
is formic acid. Since such an assumption, however, seems to be in disagreement with Harden's equation, it is rather worth while to try to explain the lack of hydrogen in *B. typhosum* fermentation as due to a reduction of acetic acid to ethyl alcohol by the hydrogen of the triose.⁹ Such a reduction of free acetic acid is, however, a strong endergonic reaction.¹⁰



The end products formed are: 1 mole of lactic acid; 1 mole of formic acid; 0.5 mole of acetic acid; and 0.5 mole of ethyl alcohol. Both qualitative and quantitative figures agree with the experimental data.

B. Different kinds of fermentations

Some soil bacteria, *Aerobacter aerogenes*, contain an enzymé which catalyzes the condensation of acetaldehyde to a carbinol which, replacing aldehyde as hydrogen acceptor, is reduced to a glycol (107).



Thus the glycol replaces the ethyl alcohol as end product in the *aerogenes* fermentation.

Some strains of *aerogenes* are also able to reduce glycerol to trimethylene glycol, a reduction which very few bacteria are able to carry out. Braak (29) in Kluyver's laboratory discovered this interesting reduction from the observation that strains of *aerogenes* were able to grow very well on glycerol as the only carbon source in the absence of oxygen. Braak demonstrated a dismutation of glycerol by isolating the reduction product, trimethylene glycol, $\text{CH}_2\text{OHCH}_2\text{CH}_2\text{OH}$, in a high yield.

⁹ Cf. the reduction of butyric acid to butanol.

¹⁰ Perhaps a phosphorylation of the carboxyl group must always precede the reduction of the carboxyl group (299, 220, 175).

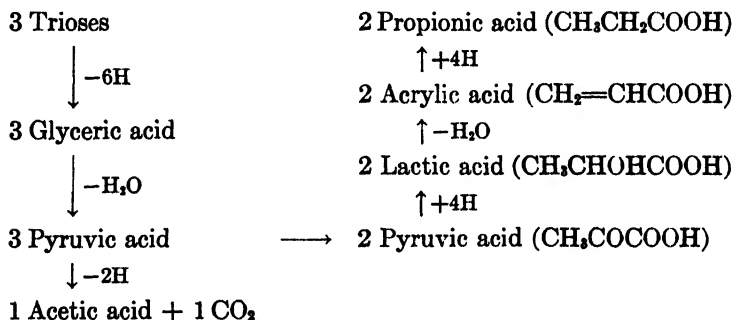
Presumably an anhydride of glycerol is formed and acts as hydrogen acceptor for glycerol. Thus acrolein is formed, together with trimethylene glycol, from glycerol by some microorganisms (287, 215).

In *propionic acid fermentation* no secondary cleavage of pyruvic acid takes place. The difference from the lactic acid fermentation is that in the propionic acid fermentation the substrate (sugar or glycerol) is oxidized two steps instead of one and, correspondingly, the acceptor (pyruvic acid) is reduced two steps, yielding propionic acid.

Fitz found the following equation for the conversion of carbohydrates into propionic acid:



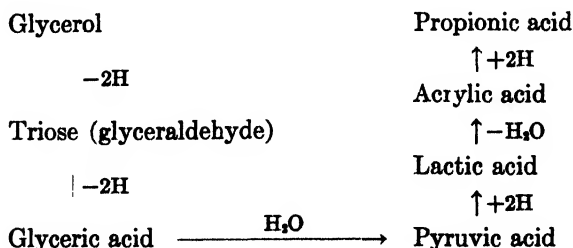
The following scheme is supported experimentally by the investigations of van Niel (227), Virtanen (285), and others:



Eight hydrogen atoms (6 + 2) are removed in dehydrogenations and eight hydrogen atoms (4 + 4) are added in reductions. Two anhydrides, pyruvic acid (the anhydride of glyceric acid) and acrylic acid (the anhydride of lactic acid), act as hydrogen acceptors.

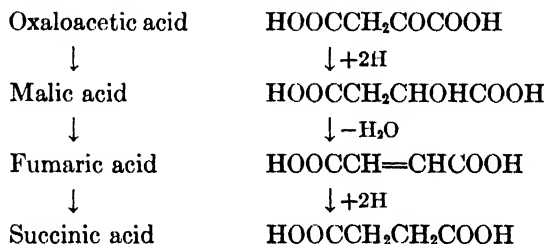
van Niel made the important observation that glycerol is converted quantitatively into propionic acid by propionic acid bacteria.

Applying the same principles as in the other fermentations, this fact can be interpreted by the following scheme:



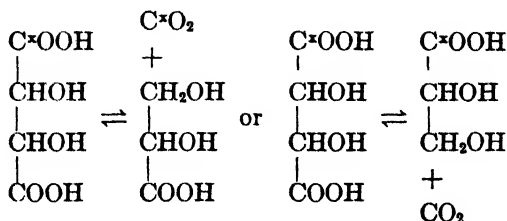
The fermentation of glycerol to propionic acid is very closely related to the fermentation of sugar to lactic acid; the main difference is that in the first fermentation a two-level oxidation and a two-level reduction ($\pm 4H$) is involved, in the last only a one-level oxidation and reduction ($\pm 2H$). Both fermentations are pure dissimilations.

Wood and Werkmann (306, 307), investigating the fermentation of glycerol to propionic acid, made the fundamental observation that the addition of carbonate caused a considerable formation of succinic acid and a corresponding decrease both in the amount of propionic acid formed and in the carbonate. This might be explained by assuming that carbon dioxide is taken up by the pyruvic acid to form oxaloacetic acid¹¹ (the reverse reaction being well known), which is then reduced instead of pyruvic acid:

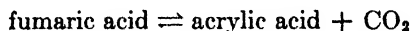


Recent experiments by Carson and Ruben (34) with radioactive carbon dioxide (C^{14}O_2) show interesting new phenomena in the propionic acid fermentation. These investigators found that if radioactive carbonate is added to propionic acid bacteria in the presence of glycerol, radioactive carbon enters into not only the succinic acid formed but also the propionic acid.

This observation indicates a reversible uptake and liberation of carbon dioxide between a C_3 -monocarboxylic acid and a symmetrical C_4 -dicarboxylic acid, for instance:

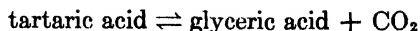


Carson and Ruben did not find an interchange between succinic acid and propionic acid; however, the reversible reaction



¹¹ Cf. also Wood *et al.* (308).

or



represents other possibilities which deserve attention.

Carson and Ruben find the ratio of the radioactivity of volatile acids to that of non-volatile acids equals approximately 3. Wood and Werkmann (307) find that the ratio propionic acid/succinic acid is 5 in the first three days of the fermentation of glycerol in the presence of carbonates; after the fifth day this ratio decreases to 4. In Carson and Ruben's experiments the incubation period was only about 30 min. and the ratio propionic acid/succinic acid formed is very likely 5 or larger. Since the ratio of the radioactivity of volatile acids/non-volatile acids is 3, the radioactivity in propionic acid is only about 50 per cent of that of succinic acid; this is in fair agreement with the hypothesis of the reversible interchange of carbon dioxide between a C_3 -monocarboxylic acid and a symmetrical C_4 -dicarboxylic acid. Wood *et al.* (308) have recently shown that the carbon isotope goes only into the carboxyl group of the C_4 -dicarboxylic acid. The recent experiments of Carson and Ruben show also that C^{14} enters only the carboxyl carbon of propionic acid.

There is reason to believe that Wood and Werkmann's carboxylation process also takes place in the fermentation of sugar to propionic acid, but since carbon dioxide is formed in this process an uptake of carbon dioxide is not easy to demonstrate. Wood and Werkmann's discovery is, furthermore, in agreement with the fact that the normal potential (calculated from thermal data) of the system

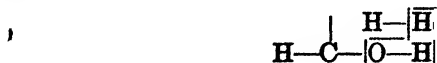


is approximately the same as that of the system



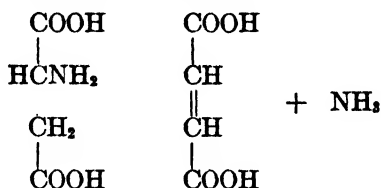
i.e., about 430 millivolts more positive than the hydrogen electrode (see table 1).

The direct experimental demonstration of acrylic acid as a hydrogen acceptor in the propionic acid fermentation is, however, still the missing link. Furthermore, the demonstration of an enzyme like fumarase which is able to attach water to acrylic acid or to remove water from lactic acid is still lacking. A direct reduction of the hydroxyl group in lactic acid, giving propionic acid, is possible and is known in organic chemistry but requires drastic treatment. The reduction of a hydroxyl group involves cleavage:



Aside from the reduction of peroxides, reductive cleavage is observed in a few cases in biological processes: e.g., the reduction of S—S compounds to 2SH or the reductive cleavage of the proline ring in *Clostridium sporogenes* (265).

The reduction of glycine and other amino acids to fatty acids (Stickland) with the liberation of ammonia is of particular interest in this connection. Is ammonia liberated before the reduction, in analogy to an anhydride formation, or is ammonia liberated during the reduction by a cleavage? The first possibility is realized in the demonstration of aspartase (246, 286), an enzyme catalyzing the reaction

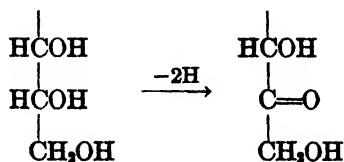


The equilibrium malic acid \rightleftharpoons fumaric acid and the replacement of propionic acid by succinic acid in the presence of carbonate are two facts which make it very desirable to find the corresponding equilibrium lactic acid \rightleftharpoons acrylic acid. Some old experiments of Dakin (56) indicate that such a reaction actually exists.

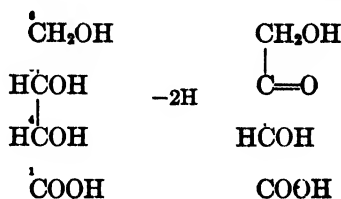
The formation of butyric acid from sugar by *Vibron butyrique* (Pasteur) is discussed in the section dealing with the formation of fatty acids from carbohydrates.

The so-called aerobic ("oxidative") fermentations (20) are respirations rather than fermentations, since oxygen is the hydrogen acceptor. To this group belong the following: acetic acid "fermentation" (i.e., more or less complete accumulation of acetic acid formed by oxidation of alcohol), and citric and fumaric acid "fermentations" (the latter two occurring in molds).

The acetic acid bacteria are able to oxidize not only ethyl alcohol but also several polyalcohols: e.g., glycerol to dihydroxyacetone and sorbitol to sorbose (22). An explanation of why keto sugars are formed was given by Bertrand, who demonstrated the importance of a definite configuration of the alcohol groups.



This phenomenon was later illustrated by Kluver and Leeuw (136), who were able to isolate the calcium salt of 5-ketogluconic acid in the oxidation of gluconic acid by an acetic acid bacterium ("Vinegar bacterium").

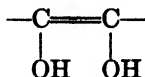


The oxidation of α -gluconic acid to 2-ketogluconic acid, which spontaneously is converted into carbon dioxide and a pentose, has also been observed (135). This last reaction is similar to the oxidation of 6-phosphohexonic acid to carbon dioxide¹² and ribose phosphate, a reaction observed in yeast juice by Lipmann (171) and later by Warburg (296) and Dickens (59).

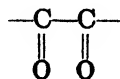
Space does not permit a discussion of the mechanism of citric acid formation in molds, although this reaction is of general interest because of the important rôle which citric acid seems to play in animal tissue respiration.

IV. THE MECHANISM OF HYDROGEN (OR ELECTRON) TRANSFER

Hopkins, Euler, and other investigators have for a long time been aware that the transfer of hydrogen ($e + \text{H}^+$) from donor to acceptor does not take place directly but requires an electron-transfer system. Such electron-transfer systems capable of taking up and giving off electrons rapidly have also been observed and isolated. Hopkins discovered the tripeptide glutathione; in 1927 Szent-Györgyi isolated ascorbic acid, which was identified as vitamin C by King and by Szent-Györgyi. Hopkins' glutathione contains an —SH group which, when oxidized one step (minus ($e + \text{H}^+$)), dimerizes to the meriquinone S—S. Ascorbic acid, which is a dienol,



is oxidized to a diketone



¹² The formation of carbon dioxide is due to a spontaneous decarboxylation of 2-ketophosphogluconic acid.

Szent-Györgyi and coworkers later described the stimulation of respiration by traces of members of the fumaric acid system. None of these substances, however, was identical with the essential electron-transfer system in fermentation and respiration.

Harden and Young in 1905 (108, 109) separated a heat-stable factor of low molecular weight from the enzyme system of alcoholic fermentation; this factor was called the coenzyme. Meyerhof in 1918 (196) demonstrated that coenzymes could also be separated from respiration enzymes (dehydrogenases). Later, when separate steps in the fermentations were described (Neuberg, Embden); the coenzyme of the fermentation was separated into different factors. Lohmann (179) found that magnesium ion and adenylic acid (adenine pentose monophosphate), a substance which Embden and Zimmermann (67) isolated from muscle tissue, are the constituents of the coenzyme of the enzymes catalyzing phosphate transfer (see later). Auhagen (5) separated the coenzyme of the enzyme which catalyzes decarboxylation from pyruvic acid (Neuberg's carboxylase). Auhagen's coenzyme was called cocarboxylase. Euler and Myrbäck (76, 77) separated another coenzyme which was a necessary component in the oxidoreduction of the alcoholic fermentation; this coenzyme was called cozymase. They purified the cozymase to a high extent and demonstrated that it contains adenylic acid but that it is not identical with adenylic acid since it contains other nitrogenous compounds (71).

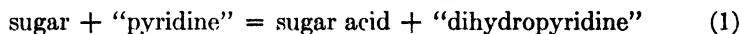
Although Euler was able to demonstrate the need of cozymase for the oxido-reduction, the nature of the action remained obscure. Cozymase occurs in all kinds of tissues, although only in very small amounts; the substance seemed to be a complicated and labile substance, difficult to separate from other nucleotides.¹³

In the course of three years (1932-35) Warburg and his coworkers succeeded not only in isolating two of the most important codehydrogenases as pure substances and in clarifying the complete constitution of one of them, but they furthermore demonstrated that the action of these coenzymes is a transfer of hydrogen (electrons) and were even able to prove what part of the complicated molecule is involved in the biological transfer of hydrogen. Besides this brilliant work, Theorell in 1935 and Negelein in 1936 in Warburg's laboratory demonstrated that the so-called coenzymes combine reversibly with specific proteins; some of these proteins have been purified by Negelein to an extent which can only be compared with the crystalline enzymes isolated by Sumner and Northrop and Kunitz.

¹³ A nucleotide is a compound built up of a nitrogen base, a sugar or an alcohol, and phosphoric acid. The name "nucleotide" is derived from the high concentration of these compounds in the nucleus of the cell (nucleic acids, nucleoproteins), particularly in the chromosomes.

A. Pyridine nucleotides, the electron-transferring component of respiration and fermentation

By 1934-35 Warburg and Christian (300) had finished their purification of the so-called respiration coenzyme ("Atmungs Coferment"). The chemical analysis showed that the coenzyme was a dinucleotide, containing the two nitrogen bases adenine and nicotinic acid amide (amide of pyridine- β -carboxylic acid), besides two sugar molecules (presumably riboses, according to Euler (71)) and three phosphates. Warburg and Christian, on the basis of the constitutional formula of the coenzyme, introduced the chemical name "Triphosphopyridine nucleotide" (the more correct name "triphosphopyridine-adenine nucleotide" seems too long). The essential constituent of the nucleotide is the pyridine derivative, a nitrogen base which at that time was completely new in enzyme biology. Warburg and Christian were able to demonstrate, partly by means of classical chemical methods and partly by ultraviolet spectroscopic methods, developed by Haas (100) in Warburg's Institute, the following fundamental reaction:



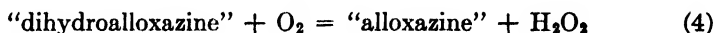
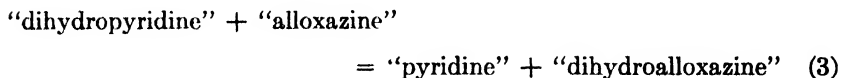
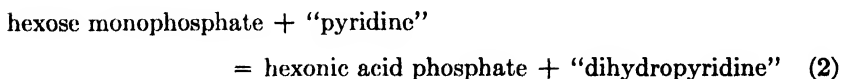
the sugar being hexose monophosphate; the "pyridine" being the triphosphopyridine nucleotide and its specific protein (in the old nomenclature called hexosemonophosphate dehydrogenase).

Negelein and Haas (221) found that the triphosphopyridine nucleotide combines with a protein specific for the substrate, in this case hexose monophosphate ("Robison ester"). This pyridine-protein is designated by Warburg as "Triphosphopyridinproteid" (Robison ester).

Warburg and Christian (296) have isolated another protein which, combined with the pyridine nucleotide, catalyzes the oxidation of a sugar acid, phosphohexonic acid, to phosphoketohexonic acid (*cf.* 171, 59). This enzyme is called "pyridine-proteid (phosphohexonic acid)"; the old term would be "phosphohexonic acid dehydrogenase".

The amount of metabolite oxidized by "pyridine" in equation 1 depends of course on the amount of "pyridine"; the reaction is actually stoichiometric. Warburg and Christian demonstrated that in the presence of oxygen and another hydrogen-transfer system, which is able to react in the oxidized form with dihydropyridine and in the reduced form with oxygen, the hydrogen from the metabolite is transferred to oxygen and in this case only extremely small amounts of the hydrogen-transferring substances are necessary for a rapid oxidation of large amounts of the metabolite. The system which is able to take hydrogen from "dihydropyridine" and give hydrogen to oxygen has also been isolated by Warburg and Christian and is called the yellow respiration enzyme or, using a more chemical terminology, the alloxazine-nucleoprotein (*see later*).

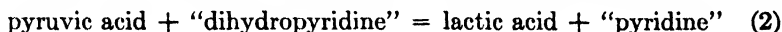
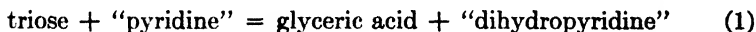
The catalytic oxidation of hexose monophosphate to phosphohexonic acid by the two nucleotide proteins can be characterized by the following three equations:



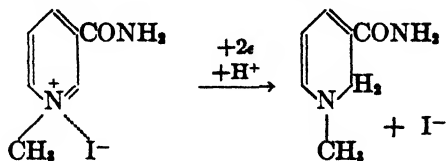
In equation 1, hexose monophosphate and "pyridine" were used up, the former as a hydrogen donor, the latter as a hydrogen acceptor. In the presence of both nucleotide proteins, even in minute amounts, only hexose monophosphate and oxygen disappear.

The coenzyme of the oxidoreduction in the fermentation, Euler's cozymase, is also a pyridine-proteid (diphosphopyridine nucleotide) transferring hydrogen from sugar to acetaldehyde and pyruvic acid. This was demonstrated at the same time by Warburg and Christian (295) and by Euler, Albers, and Schlenck (75). The only difference from the other pyridine compound is, as shown by Warburg and Christian, that the hydrogen transfer from triose phosphate through the diphosphopyridine nucleotide to pyruvic acid depends on the presence of inorganic phosphate and adenine nucleotide (adenosine diphosphate). This phosphate effect will be discussed in the next section.

In glycolysis the diphosphopyridine nucleotide transfers hydrogen from triose (as the phosphate ester) to pyruvic acid:

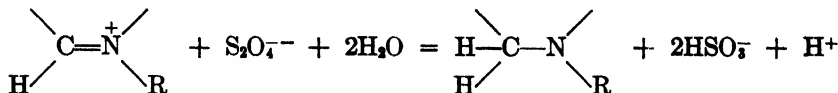


Warburg and coworkers, having proved that the pyridine ring is the hydrogen-transferring system, started, in collaboration with Karrer (125), to find out whether the C=C or C=N double bond is the carrier of hydrogen removed from the metabolites. Karrer (124) synthesized the iodomethylate of nicotinic acid amide and, by reducing this substance with hydrosulfite, established the following reaction:



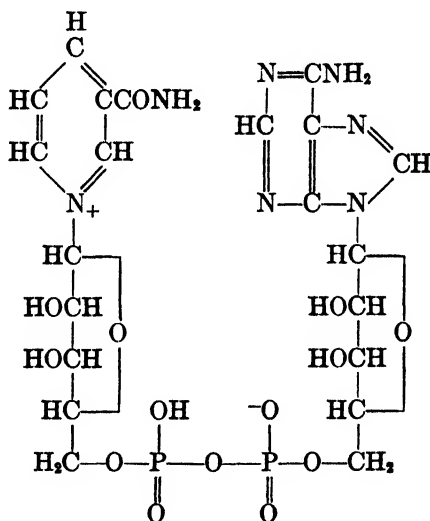
By reduction with sodium hydrosulfite ($\text{Na}_2\text{S}_2\text{O}_4$) a new acid equivalent (HI) arises besides sodium bisulfite. The $\begin{array}{c} \diagup \\ \text{C}=\text{N}^+ \\ \diagdown \end{array}$ group is therefore the oxidized form and the $\begin{array}{c} \diagup \\ \text{C}-\text{N} \\ \diagdown \end{array}$ group the reduced form.

Warburg and Christian (295, 297) then demonstrated that reduction of the pyridine nucleotides with hydrosulfite gives rise to an extra acid group, owing to the removal of the quaternary nitrogen:

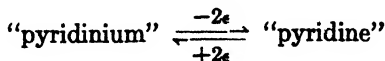


The extra acid will be taken up by the phosphate anion. Thus the pyridine nucleotides are quaternary nitrogen bases (pyridinium derivatives).

Furthermore, investigations of Euler and coworkers (78, 283) showed that alkali inactivation of the pyridine nucleotides yields adenosine diphosphate. On the basis of their investigations, Euler (71) illustrated the oxidized and reduced diphosphopyridine nucleotide in the following manner:

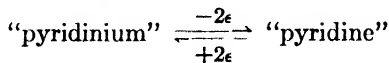


The redox potential of the system



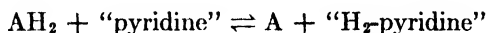
is not exactly known. Clark (35), however, has calculated the potential in the presence of the pyridine-proteid. He obtained E'_0 (pH 7) = -0.250

volt. Ball (6), using Clark's indicators and an enzyme system from milk, estimated the potential of the



system at E'_0 (pH 7) = -0.260 volt. Borsook (25) recently calculated E'_0 from the detailed and careful equilibrium investigations of Euler and coworkers (glutamic acid-ketoglutaric acid system) and found E'_0 (pH 7) to be -0.280 volt. This potential is from 180 to 150 millivolts more positive than the hydrogen electrode but is rather negative in comparison with the alloxazine system and particularly with the hemin system. Most of Clark's indicators (dyes) have potentials more positive than the pyridine system. The γ, γ -dipyridyl dyes, however, have potentials more negative than the pyridine system; E'_0 (pH 7) for the dipyridyl dye methyl viologen is even 20 millivolts more negative than the hydrogen electrode.

Negelein and coworkers (221, 222) were able to isolate in a very pure state some of the proteins which are necessary for the pyridine catalysis. They furthermore showed, by means of very exact kinetic measurements, using the spectrophotometric method of Haas, that the pyridine nucleotide and the protein combine to a proteid able to dissociate. The reduced pyridine nucleotide ("dihydropyridine") forms a dissociable compound with the same protein ("dihydropyridine-proteid"). The pyridine-proteid is the compound which catalyzes the oxidation of the metabolite (or the reduction of "pyridine"). The velocity of this electron transfer is therefore proportional not only to the total amount of protein (E) but also to the ratio pyridine-proteid/proteids + protein (ρ_{ox}). On the basis of the reaction



calling the concentrations of "pyridine" and "H₂-pyridine" C_{ox} and C_{red} , the velocity of the reaction is:

$$\frac{dC_{ox}}{dt} = k \cdot E \cdot \rho_{ox}$$

where k is a velocity constant. If the reaction is reversible, the expression is:

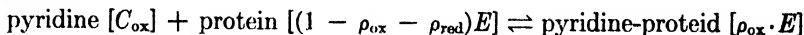
$$\frac{dC_{ox}}{dt} = k_{ox} \cdot E \cdot \rho_{ox} - k_{red} \cdot E \cdot \rho_{red}$$

where k_{ox} is the velocity constant for the reaction from left to right and k_{red} for the reaction from right to left.

Considering the simple case where the reaction is only going from left

to right, Warburg illustrates the kinetics of the enzymatic oxidation-reduction in the following manner: In order to understand the nature of the enzymic pyridine reduction better, ρ_{ox} is expressed by the dissociation constants of the pyridine-proteid (D_{ox}) and of the dihydropyridine-proteid (D_{red}).

The dissociation of the pyridine-proteid is expressed in the following equation:



The dissociation constant (D_{ox}) for this reaction is

$$D_{ox} = \frac{[C_{ox}] \cdot [(1 - \rho_{ox} - \rho_{red})E]}{[\rho_{ox} \cdot E]}$$

The dissociation constant of the dissociable dihydropyridine-proteid (D_{red}) is

$$D_{red} = \frac{[C_{red}] \cdot [(1 - \rho_{ox} - \rho_{red})E]}{[\rho_{red} \cdot E]}$$

From these two equations ρ_{ox} is obtained:

$$\rho_{ox} = \frac{C_{ox} \cdot D_{red}}{C_{ox}(D_{red} - D_{ox}) + D_{ox}(C + D_{red})}$$

where $C = C_{red} + C_{ox}$, i.e., the total concentration of nucleotide. The equation for ρ_{ox} used in the velocity equation gives:

$$\frac{dC_{ox}}{dt} = k \cdot E \cdot \frac{C_{ox} \cdot D_{red}}{C_{ox}(D_{red} - D_{ox}) + D_{ox}(C + D_{red})}$$

These formulas illustrate the importance of D_{red} for the velocity of the reaction from left to right. Increasing D_{red} , which means easier removal of "dihydropyridine" from the protein, increases the velocity of the reaction from left to right ($-dC_{ox}/dt$). In the "pyridine-proteid (hexose monophosphate)," $D_{ox} = D_{red}$, which gives the simple equation:

$$-\frac{dC_{ox}}{dt} = C_{ox} \left[\frac{k \cdot E}{C + D} \right]$$

If the total concentration of nucleotide (C) is great in relation to D , the velocity equation is even simpler:

$$-\frac{dC_{ox}}{dt} = C_{ox} \left[\frac{k \cdot E}{C} \right]$$

If C is in great excess, the velocity of the reaction at $t = 0$ (i.e., $C_{ox} = C$) depends only on the total amount of protein:

$$-\frac{dC_{ox}}{dt} = k \cdot E$$

The independence of C indicates that the protein is completely saturated with "pyridine."

If $C = D$ the velocity at $t = 0$ is

$$\frac{dC_{ox}}{dt} = C \frac{kE}{2C} = \frac{kE}{2}$$

which means that the dissociation constant (D) is equal to that nucleotide concentration which saturates the protein (E) to the extent of 50 per cent (moles nucleotide > moles protein).

Negelein and coworkers have obtained excellent agreement between the theory and the experiments, which thus establishes the suggestion that the rate of oxidation of metabolites by "pyridine" and a special protein depends on the concentration of the dissociable compound: "pyridine"-protein.

The rate of reduction of "pyridine" per unit of coenzyme is constant most of the time, provided that C_{ox} is great enough to saturate the protein completely. If the nucleotide concentration is increased to a very high extent the rate per milligram of nucleotide decreases, i.e., the efficiency decreases.

Negelein and Wulff (222) found that the protein catalyzing the oxidation of ethyl alcohol to acetaldehyde (or the reverse process) is 50 per cent saturated at a concentration of "dihydropyridine" one-third that of "pyridine." Furthermore, the catalytically active proteins form dissociable compounds with the substrates which are being oxidized or reduced. The idea of a substrate-enzyme compound was advanced as early as 1913 by Michaelis and Menten (209) on the basis of their studies of invertase action and has been very useful in the interpretation of enzyme kinetics.

I shall here present some of Negelein's values for the dissociation constants of protein-nucleotide compounds and for protein-substrate compounds.

	<i>D</i> OF PYRIDINE NUCLEOTIDES, THE SO-CALLED PROSTHETIC GROUP OF "PYRIDINE-ENZYMES" (D_{ox})	<i>D</i> OF DIHYDROPYRIDINE NUCLEOTIDES, THE SO-CALLED PROSTHETIC GROUP OF REDUCING "PYRIDINE-ENZYMES" (D_{red})
Hexosemonophosphate dehydrogenase	1.1×10^{-5}	$1 \times 10^{-5} \left[\frac{\text{moles nucleotide}}{\text{liter}} \right]$
Alcohol dehydrogenase	9.5×10^{-5}	$3.2 \times 10^{-5} \left[\frac{\text{moles nucleotide}}{\text{liter}} \right]$

The D values of the alloxazine nucleoproteins are at least one hundred times smaller than the D values of the pyridine nucleoproteins.

The dissociation constants of some coenzyme-enzyme complexes are presented below:

COENZYME-ENZYME COMPLEX	DISSOCIATION CONSTANT	REFERENCES
Alcohol dehydrogenase (diphosphopyridine-protein)	9.0×10^{-8}	(222)
<i>D</i> -Amino acid oxidase (flavinadenine-protein)	2.5×10^{-7}	(219)
Pyruvic acid dehydrogenase (thiaminpyrophosphate-protein)	2.7×10^{-8}	(175)
Glycogen phosphorylase (adenylic acid-glycogen-protein)	3.0×10^{-8}	(51)

In the equation

$$-\frac{dC_{ox}}{dt} = C \frac{kE}{2C} = \frac{kE}{2}$$

the velocity constant k can be calculated.

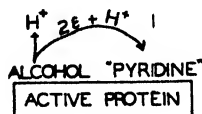
In the oxidation of hexose monophosphate by a "pyridine-proteid", $k_{(ox)} = 2.9 \times 10^4$ (1 min.), which means that 1 molecule of specific protein in 1 min. is able to bring 29,000 molecules of hexose monophosphate and pyridine nucleotide to reaction (221).

The velocity constant of the oxidation of alcohol by a "pyridine-proteid" is $k_{ox} = 1.7 \times 10^4$ (1 min.) (222).

The velocity constant of the reduction of acetaldehyde by a "dihydropyridine-proteid" is (222)

$$k = 2.9 \times 10^4 \text{ (1 min.)}$$

All these studies have shown that the oxidation-reduction nucleotides and ordinary substrates display two properties in common: (1) hydrogen is transferred from the substrate to the nucleotide by an ordinary stoichiometrical reaction, and (2) both the substrate and the nucleotide form dissociable compounds with the catalytically active protein, e.g.:

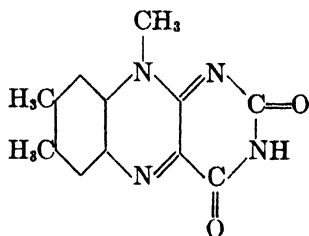


Owing to these facts, several biochemists prefer to classify the nucleotides as substrates. Several substrates like the fumaric acid system (271) and amino acids (140) are actually able to act as e -transfer systems like the nucleotides. Although the analogies between substrates and nucleotides are of much importance, Warburg is justified in laying stress on the important differences between ordinary substrates and the nucleotides. The

alloxazine nucleotides and thiazole nucleotides (175), for instance, form proteids which (at pH 7) have dissociation constants far smaller than the substrate proteids. Another feature characteristic of the system of oxidation reduction nucleotides is the appearance of semiquinones (210, 101), a phenomenon which will be discussed at the end of this section.

B. Alloxazine nucleotides

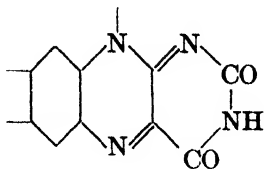
In 1932 Warburg and Christian (293) isolated a yellow enzyme protein compound which was decolorized by reduction; when the decolorized enzyme was reoxidized the yellow color returned. In alkaline solution and in light, the yellow dye was converted into another yellow dye which was soluble in chloroform; this substance was called lumiflavin. Warburg and Christian (294) isolated the lumiflavin ($C_{13}H_{12}N_4O_2$) and showed that urea is liberated by its alkaline hydrolysis. Stern and Holiday (263) showed that lumiflavin was an alloxazine with a methylated nitrogen, and Kuhn and coworkers (156) established that lumiflavin is trimethylalloxazine:



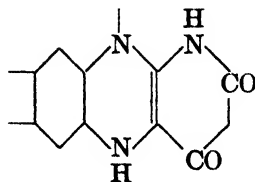
Lumiflavin

Hydrogen on a platinum catalyst reduces the lumiflavin to dihydroflavin, which is colorless; oxygen oxidizes the dihydroalloxazine to the yellow lumiflavin (294).

Warburg and Christian (294) obtained detailed absorption spectra for the yellow enzyme and for lumiflavin. The two spectra possess the essential resemblance. Since the spectrum of the yellow enzyme is an alloxazine spectrum, the hydrogen transfer by the yellow enzyme can be described as an action of the conjugated $C=N$ units:



Alloxazine

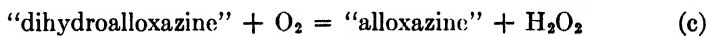
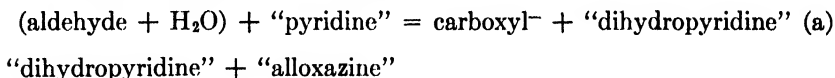


Dihydroalloxazine

Kuhn and coworkers (157) in 1933-34 found that lumiflavin is a cleavage product of an alloxazine-ribityl compound (riboflavin), and in 1934 Theorell (275) discovered that the prosthetic group of the yellow enzyme is alloxazine-ribityl phosphate (riboflavin phosphate), i.e., a nucleotide. Kuhn and coworkers (1936) (154) succeeded in synthesizing the riboflavin phosphate.

Theorell in 1934 (276) succeeded in separating the alloxazine nucleotide and the specific protein without irreversible denaturation of the protein by dialysis for 72 hr. against cooled dilute hydrochloric acid; addition of alloxazine nucleotide to the protein gave resynthesis of the yellow enzyme. This was the first separation and reactivation of a respiration enzyme.

The alloxazine-proteid transfers electrons from dihydropyridine nucleotides to oxygen, thus completing systems which oxidize aldehydes to acids or alcohols to aldehydes by oxygen:



Like the pyridine nucleotides, the free alloxazine nucleotide is inactive; only the alloxazine nucleoprotein is active.

Theorell (279) proved that at low oxygen pressure the "dihydroalloxazine" can be reoxidized by the ferric ion of cytochrome *c*. F. G. Fischer (82) and Szent-Györgyi and coworkers (272, 158) showed that "dihydroalloxazine" can be reoxidized by fumaric acid in the presence of special enzymes.

In 1938 Warburg and coworkers (298) isolated some new yellow enzymes, the prosthetic group of which they showed to be alloxazine-adenine dinucleotides. These alloxazine dinucleotides have a striking resemblance to the constitution of pyridine-adenine nucleotides. A number of these new alloxazine-proteids were isolated at the same time in Warburg's Institute and by Straub (266) in Keilin's Institute. The experiments of Warburg and coworkers and those of Straub show that these new alloxazine-proteids are considerably more active than the old enzymes isolated in 1932. Combined with different proteins, the alloxazine dinucleotides are able to react not only with the pyridine nucleotide and with amino acids (298) but also with other systems; Corran, Green, and Straub (54) showed that one of the alloxazine-proteids isolated by Straub from heart muscle transfers hydrogen from "dihydropyridine" to methylene blue (and probably also to cytochrome) with an enormous velocity. Warburg points out that the

"old" alloxazine nucleotides might be cleavage products of the "new" dinucleotides.^{12a}

Warburg and Christian (298) in their new research on the yellow enzyme introduced a very convenient method for the separation of alloxazine nucleotides from alloxazine nucleoproteins which have a very small dissociation constant: addition of *m*/10 hydrochloric acid to a cooled solution of the flavoprotein in 20 per cent ammonium sulfate solution liberates the alloxazine group, the ammonium sulfate protecting the protein against acid denaturation. Recombination experiments gave an excellent yield: 78 per cent of the protein and 95 per cent of the alloxazine dinucleotide remained active during the separation (298). The alloxazine nucleoproteins are dissociable only to an exceedingly small extent. The alanine

TABLE 2
Systems which reduce and oxidize alloxazine

REDUCING SYSTEM	ALLOXAZINE PROTEID SYSTEM	OXIDIZING SYSTEM
Triphosphopyridine nucleotide (malic acid)	Alloxazine mononucleotide (Warburg and Christian, 1932)	Oxygen Cytochrome <i>c</i> (Theorell) Fumaric acid (Szent-Gyorgyi)
<i>d</i> -Amino acids (<i>d</i> -alanine, <i>d</i> -proline, cysteine)	Alloxazine dinucleotide (Warburg and Christian, 1938)	Oxygen
Diphosphopyridine nucleotide	Alloxazine dinucleotide (Haas, 1938)	Methylene blue
Diphosphopyridine nucleotide	Alloxazine dinucleotide (Straub, 1938)	Methylene blue (cytochrome?)
Triphosphopyridine nucleotide	Alloxazine dinucleotide (Warburg and Christian, 1938)	Oxygen
Thiamin nucleotide (Lipmann, 1939)	Alloxazine dinucleotide (F. G. Fischer, 1939)	Oxygen Fumaric acid

"oxidase" has the highest dissociation constant: $K = 2.5 \times 10^{-7}$ moles per liter. A number of catalytically active alloxazine-proteids have been isolated; table 2 summarizes what is known about the systems which reduce and oxidize alloxazine.

Furthermore Ball (7), in Warburg's Institute, purified the xanthine "oxidase" and found that the alloxazine dinucleotide is one of the prosthetic groups of this enzyme. A similar observation was made by Gordon Green and Subrahmanyam (94) in purifying an aldehyde dehydrogenase from liver tissue. The xanthine oxidase and the aldehyde oxidase con-

^{12a} Very recently, Haas, Horecker, and Hogness (J. Biol. Chem. **136**, 747(1940)) in some brilliant studies discovered a new, very active yellow enzyme, the prosthetic group of which is an alloxazine mononucleotide. The new yellow enzyme transfers electrons from triphosphopyridine nucleotide to cytochrome *c*.

tain another coenzyme besides the flavin component; the second coenzyme has not yet been identified.

Franke and Deffner (87) recently obtained a purified glucose dehydrogenase, the activity of which was found to be proportional to the content of alloxazine.

The normal redox potentials of free alloxazine nucleotides (211) amount to about -150 millivolts (pH 7), whereas the alloxazine-proteids have higher potentials, about -80 millivolts. The redox potential of the alanine "oxidase" is not known, but since the dihydroalloxazine forms a proteid which experimentally is undissociable, the potential will be raised to a considerable extent (*cf.* 36, 57).

It is well known that Warburg's discoveries of nicotinic acid and alloxazine as the essential compounds in redox enzymes were of fundamental importance for the numerous investigations of the action of these substances on growth. The result of these investigations was the identification of previously unknown growth factors (vitamins) with these two compounds.

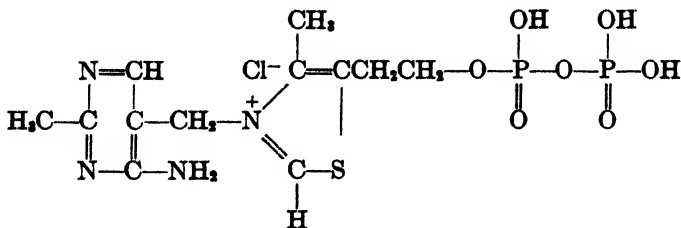
C. Thiamin nucleotides

The antiberiberi factor, vitamin B₁ (or ancurin), was identified by the work of Williams and of Windaus as a thiazole-pyrimidine derivative. In the case of B₁ the vitamin function was known long before the enzyme action.

Experiments of Peters and coworkers (244) showed that brain tissue from pigeons which have beriberi symptoms has a smaller oxygen consumption than normal brain tissue and exhibits an accumulation of acids. Peters and collaborators identified the acid accumulated in beriberi tissue as pyruvic acid and were furthermore able to demonstrate that lack of vitamin B₁ stopped or inhibited the further breakdown of pyruvic acid.

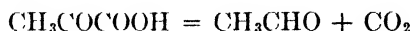
As mentioned before, the enzyme which catalyzes the decarboxylation of pyruvic acid to acetaldehyde and carbon dioxide, Neuberg's carboxylase, is a protein compound from which a prosthetic group, the so-called cocarboxylase, can be liberated (5).

In 1937 Lohmann and Schuster (184) identified Auhagen's cocarboxylase as vitamin B₁ pyrophosphate (thiamin pyrophosphate):



Thiamin nucleotide

Lohmann and Schuster resynthesized carboxylase by addition of thiamin pyrophosphate to the specific protein; thus they established that Neuberg's carboxylase, the enzyme which catalyzes the reaction



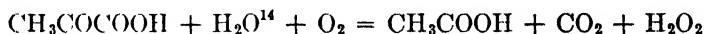
is a thiamin nucleoprotein

Krebs (147, 148), as well as Lipmann (173), discovered a new type of reaction, the dismutation of pyruvic acid:



One molecule of pyruvic acid acts as hydrogen acceptor, the other together with water¹⁴ as hydrogen donor, the first yielding lactic acid, the second acetic acid and carbon dioxide.

Lipmann (174) furthermore demonstrated that pyruvic acid can be oxidized in animal tissue as well as by lactic acid bacteria according to the equation:



He was able to obtain the pyruvic acid dehydrogenase from dried lactic acid bacteria in a stable form. Treating this concentrated enzyme preparation in the same manner as Lohmann did, Lipmann inactivated the system and reactivated it by addition of very small amounts of Lohmann's pure thiamin pyrophosphate. Recently (176) he observed that a purified pyruvic acid dehydrogenase treated according to the method of Warburg and Christian (dilute hydrochloric acid in the presence of ammonium sulfate) is inactivated. Apparently the dehydrogenase contains another component besides thiamin phosphate. Lipmann was able to restore the pyruvic acid dehydrogenase with a small amount of Warburg's alloxazine dinucleotide and thiamin pyrophosphate. The pyruvic acid dehydrogenase therefore is composed of a substance of high molecular weight (presumably a protein) and two prosthetic groups, thiamin and alloxazine nucleotides; the thiamin system therefore presents a striking resemblance to the pyridine-alloxazine system. Lipmann's experiments furthermore show that phosphate and adenylic acid are necessary components of the system. This last observation will be discussed in the next section.

Recently Green, Herbert, and Subrahmanyam (98) have isolated the thiamin nucleoprotein. It contains 0.46 per cent of diphosphothiamin and 0.13 per cent of magnesium. In high salt concentrations carboxylase is a firmly bound conjugated protein, whereas in dilute salt solutions or in alkaline ammonium sulfate solutions it dissociates into protein, diphospho-

¹⁴ Later discoveries of Lipmann (175) show that phosphate and not water creates the reductans proper (see section V).

thiamin, and magnesium. It is a fairly generally accepted view that the phosphate or pyrophosphate and the amino groups in nucleotides represent the structures which link the coenzymes to the specific protein. This view was originally based upon the observations of Kuhn and collaborators (154), who found that the specific protein of the yellow enzyme can act not only with the flavin phosphate but also with the unphosphorylated flavin. In the last case, however, a much larger amount of protein had to be used in order to get the same activity as with flavin phosphate. This difference in activity was explained as being due to the difference in binding groups. Flavin is bound to the protein by one group, the amino group; flavin phosphate is bound by two groups, the amino group and the phosphate group.¹⁵

The inhibition of certain dehydrogenases by phosphate has been explained as a competition between the phosphate of the active nucleotide and inorganic phosphate. The phosphate concentrations which inhibit these dehydrogenases are, however, rather large. Some recent experiments by Buchmann and Heegaard (32) are of considerable interest for this problem. These investigators worked with thiamin pyrophosphate (cocarboxylase) and the specific enzyme protein. The activation of the enzyme protein by cocarboxylase was strongly inhibited by very small amounts of thiazole pyrophosphate but not at all by free thiazole. The same investigators (112) have also been able to give evidence for the hypothesis that amino groups play an essential rôle in linking coenzymes to enzyme proteins. They showed that the activation of the carboxylase protein by cocarboxylase is inhibited strongly even by minute amounts (16 γ per cubic centimeter) of the aminopyrimidine which is a constituent of the cocarboxylase. The corresponding deaminated pyrimidine (hydroxypyrimidine) exerts even in large amounts no inhibitory effect on the reaction between cocarboxylase and the carboxylase protein.

Green *et al.* (98) are of the opinion that magnesium, which they find as a constituent of thiamin nucleoprotein (carboxylase), plays a rôle in linking the nucleotide to the specific protein.

The rôle of the quaternary nitrogen in thiamin

Lipmann, besides studying the enzymatic pyruvic acid oxidation, made an attempt to clarify the nature of the thiamin action in the oxidation of pyruvic acid. It is known that pyridine and thiazole are very closely related compounds, displaying the same physical properties. This, in connection with the above mentioned resemblance of the pyruvate oxida-

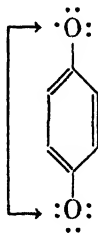
¹⁵ Recent experiments by Warburg and Christian (299) demonstrate clearly the rôle of phosphate esterified to alcohol groups: 3-phosphotriose needs 1000 times less protein catalyst than free triose in order to be oxidized to glyceric acid.

tion to triose phosphate oxidation, led Lipmann to the assumption that the quaternary nitrogen in thiamin, as in the pyridinium compounds, accepts hydrogen from pyruvic acid and transfers it to the alloxazine system. Lipmann (172, 178) was actually able to reduce the quaternary nitrogen in thiamin and thiazole derivatives by hydrosulfite but the reduced product could not be reoxidized, presumably because of a secondary cleavage reaction (cf. 70). Later attempts (14, 264) to demonstrate a reversible oxidoreduction of thiamin have only established Lipmann's findings but have not provided further contributions to this theory.

D. The rôle of specific proteins in the formation of semiquinones

A gradual reduction of pyridine, thiamin, and flavin nucleotides with sodium hydrosulfite ($\text{Na}_2\text{S}_2\text{O}_4$) always yields intermediate, strongly colored products. These colored products, which again disappear when the reduction is complete, are supposed to be semiquinones (211, 212, 172).

Michaelis and collaborators (212) have demonstrated that one-step reduction, i.e., an uptake of only one electron, yielding a free radical, actually takes place for quite a number of dyes. The best possibilities for an accumulation of semiquinones, i.e., stabilization of a free radical, are in case the molecule involved has a symmetrical configuration, as, for instance, the ion:



A structure having one electron resonating between two equivalent structures represents a close analogy to the so-called three-electron bond (Pauling (240)). Since the quinones are ionized in strong alkali, the chances for an accumulation of the free radical are best in alkaline reactions.

The corresponding nitrogen compounds are able to accumulate free radicals at very acid pH values only; this is also the case with the alloxazines.

Haas (101) in Warburg's laboratory has made the important observation that when the alloxazine nucleoprotein is reduced at 0°C . by reduced triphosphopyridine nucleotide, a transitory intermediate red product is formed. This red compound has the same absorption spectrum as the

red radical that appears when free flavin is reduced by hydrosulfite at a pH less than 0. Thus the combination with the specific protein in neutral solutions seems to have the same effect as has a strong acid, displacing the equilibrium in favor of the radical.¹⁵

Michaelis is of the opinion that such a displacement of the equilibrium in favor of the radical might be one of the most essential actions of the catalytically active proteins of redox nucleotides (214, page 6):

The following general principle may be postulated: In most organic compounds an oxidation of any valence-saturated compound to another such compound on a higher level of oxidation is a bivalent oxidation. The inertia of organic compounds toward oxidizing agents is due to the fact that the oxidation can proceed at a measurable speed only in two successive univalent steps and consequently only if the intermediate radical can be formed. If the normal potential of the first step of oxidation is much higher than that of the second step the amount of the radical formed may be extremely slight and its concentration may be the limiting factor for the speed of oxidation. In many organic compounds this situation leads to a practical lack of reactivity at ordinary temperatures in absence of a catalyst. The rôle of an enzyme, then, is to displace the equilibria concerned in favor of the radical. A possible demonstration of this is Haas' experiment cited above: the combination with a specific protein and a coenzyme displaces the equilibrium in favor of the radical. . . .

Warburg expresses his opinion of the action of the specific protein in somewhat the same direction:

Warum das Eiweiss so wirkt, ist heute das Problem der Fermentchemie. Zwei Gründe lassen sich zur Zeit anführen:

1. Wenn das Alloxazin an das Eiweiss gebunden wird, so wandert das Absorptionsspektrum der Wirkungsgruppe um 20 m μ nach rot, was bedeutet, dass die Aktivierungsenergie des Alloxazins durch die Bindung an das Eiweiss kleiner, die Reaktionsfähigkeit des Farbstoffes also grösser wird.

2. In Lösungen von Alloxazinproteid und Pyridin-Nucleotid tritt unter gewissen Bedingungen eine Farbe auf, die nur von einer Verbindung der beiden Substanzen herrühren kann. Wahrscheinlich also sind die Reaktionen zwischen Alloxazin und hydrierten Pyridin innermolekulare Proteid-Reaktionen. Dann versteht man sofort, warum die nicht an Eiweiss gebundenen Flavin mit den hydrierten Pyridin-Nucleotiden nicht reagieren.¹¹

Both statements indicate that the task of the redox enzymes may be to decrease the extraordinarily high instability of the semiquinones of ordinary metabolites, thereby increasing the concentration of the proper reactive product. The electron acceptors, double bonds, seem to be independent of catalysts because they possess a certain degree of unsaturation,

¹⁵ Pauling and Coryell (241) have found that hemoglobin contains four unpaired electrons per heme; oxyhemoglobin and carbon monoxide hemoglobin, however, contain no unpaired electrons. The oxygen molecule with two unpaired electrons in the free state accordingly undergoes a profound change in electronic structure on attachment to hemoglobin.

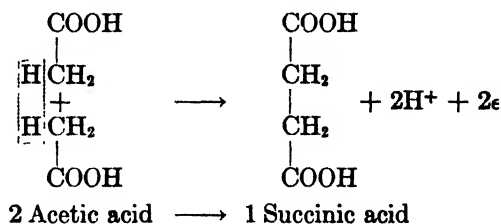
exhibiting paramagnetic properties (166). That much can be said today about the nature of specific protein catalysis in biological oxidation-reduction.

E. The fumaric acid system

Szent-Gyorgyi and collaborators (270) have shown that the dicarboxylic acids, succinic-fumaric acid and malic-oxaloacetic acid, when added to tissue systems in minute amounts (0.1 to 0.2 mg.) increase the oxygen uptake; the extra respiration exceeds several times the amount of oxygen necessary for a complete combustion of the small amount of dicarboxylic acids added. Szent-Gyorgyi therefore assumes that the dicarboxylic acids act as a hydrogen-transfer system like the nucleotides just mentioned. According to the potentials the malic acid-oxaloacetic acid system should work between the pyridine and the alloxazine system, and the succinic acid-fumaric acid system between alloxazine and cytochrome. Laki (158) showed that reactions of this kind actually take place.

Several metabolites seem, however, to be oxidized with the two nucleotides and the cytochrome system as the only hydrogen-transfer systems (279, 54). On the other hand, Annau and Erdos (4) showed that oxidation of pyruvic acid to acetic acid requires minute amounts of succinic acid (*cf.* also 8), and Colowick, Welch, and Cori (40) have recently demonstrated the importance of the succinic acid-fumaric acid system for the phosphorylation of glucose in kidney extracts.

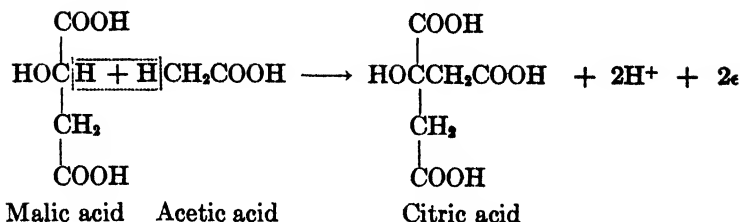
Thunberg (281), Krebs (151), and others assume that the fumaric acid system acts as carrier of acetic acid in the oxidation of this substance. An oxidative dimerization of two molecules of acetic acid gives succinic acid¹⁷ (Thunberg):



The succinic acid then is oxidized to oxaloacetic acid and this substance is spontaneously decarboxylated to pyruvic acid, which when oxidized again gives acetic acid.

If a molecule of acetic acid is oxidatively condensed with malic acid, citric acid is formed.

¹⁷ *Cf.* the oxidative dimerization of —SH compounds to S—S compounds.



Simola (258, 125) recently demonstrated a very considerable formation of citric acid from pyruvic acid and malic acid, a formation which very likely corresponds to the oxidative condensation of acetic acid and malic acid. The oxidation of acetic acid and malic acid to citric acid is presumably of importance for the formation of glutamic acid (*cf.* section VII).

F. Cytochrome and pheohemin

In 1925 Keilin (126) discovered some hemins which in the reduced form exhibit typical spectral lines. Such cell hemins were observed fifty years ago by McMunn but no attention was paid to his findings. Keilin, analyzing the cytochrome spectrum, came to the conclusion that three different cytochromes exist, which he called *a*, *b*, and *c*.

In 1936 Theorell (278) isolated cytochrome *c* as a pure substance. Cytochrome *c* is a hemin in combination with a basic protein. Recently Keilin and Hartree (127) described a very simple method for obtaining pure cytochrome *c* from heart muscle. The minced and washed heart muscle is treated with dilute trichloroacetic acid. A large amount of cytochrome *c* is liberated and can be precipitated at pH 3.5. By this method Keilin and Hartree were able to obtain the enormous yield of 1.5 g. of pure cytochrome *c* per kilogram of heart muscle.

Succinic acid seems to react directly with cytochrome *c* (273, 232). Theorell (279) showed that the reduced alloxazine nucleotide reacts directly with cytochrome *c*, giving alloxazine and reduced cytochrome *c* (*cf.* Haas *et al.* (1940)). This latter product cannot be oxidized directly by oxygen but only through Warburg's pheohemin enzyme (cytochrome oxidase; "Atmungsferment"). According to Ball (6), cytochrome *b* has the lowest potential and cytochrome *a* has the highest potential.¹⁸

G. The hemin catalysis

The hemin (iron porphyrin) catalysis was predicted by Warburg in 1923 (290) on the basis of some model experiments. Later Warburg demonstrated the occurrence of a hemin compound in yeast cells. This hemin compound is involved in the transfer of electrons from cytochrome

¹⁸ The potentials are as follows: cytochrome *a*, +290 millivolts; cytochrome *c*, +270 millivolts; cytochrome *b*, -40 millivolts.

to oxygen. The reaction with oxygen is strongly inhibited by carbon monoxide, which combines with the reduced enzyme (Fe^{++}), and by cyanide, which combines with the oxidized enzyme (Fe^{+++}). The carbon monoxide enzyme compound is split photochemically by certain wave lengths and hence is again available for oxygen. Following this principle Warburg obtained a detailed action-spectrum. This indirect spectrum was compared with the direct spectra of a number of different hemins. Warburg (291) identified the "oxygen-activating enzyme" as a pheohemin which with regard to its spectrum is an intermediate between red and green hemins. Keilin's so-called cytochrome a_3 (128) is perhaps identical with the oxygen-activating enzyme.

The importance of other heavy metals besides iron has been demonstrated in recent years. Kubowitz (152, 153) purified an enzyme which oxidizes polyphenols to quinones and showed that it is a copper protein. He furthermore showed, by the following ingenious arrangement, that copper ion is the prosthetic group of this enzyme: The copper enzyme was dialyzed against a dilute solution of cyanide which trapped the copper; the enzyme inactivated by this procedure was immediately reactivated by small amounts of copper ions.

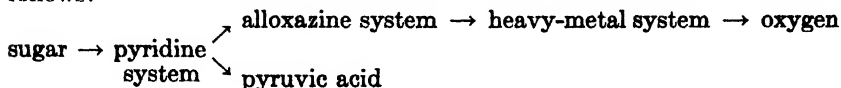
Keilin and collaborators purified other polyphenoloxidases and identified them as copper proteins. In a recent paper Keilin and Mann (129) have made the interesting discovery that ascorbic acid, which is an aliphatic dienol, is oxidized by polyphenoloxidases provided a trace (0.1 mg. per cubic centimeter) of catechol is added. Szent-Gyorgyi and collaborators (274) have recently discovered an enzyme which catalyzes the oxidation of dihydroxymaleic acid, which is a typical dienol. Swedin and Theorell (268) have purified this enzyme to a considerable degree; the enzyme seems to be a kind of peroxidase (*cf.* 267a, 268). The enzyme seems also to be a heavy-metal-protein compound. Szent-Gyorgyi is of the opinion that all dienols or diphenols form heavy-metal complexes, a suggestion which he was able to support in model experiments. A large number of dienols and diphenols actually form deeply colored iron complexes in water solution.

The heavy metals act presumably in all cases as electron-transfer systems, alternating between the ferro and ferri or cupro and cupri states.

The brilliant studies of Keilin and Mann (1940), showing that carboanhydrase is a zinc protein, should be mentioned here, although this subject is beyond the scope of this review.

H. The Pasteur reaction

The relation between fermentation and respiration can be illustrated as follows:



The alloxazine group of a yellow enzyme and the pyruvic acid compete for the reduced pyridine group; if oxygen is available, the alloxazine is kept in the oxidized form and will therefore be the strongest hydrogen acceptor. In the absence of oxygen (anerobic conditions), the alloxazine will be completely reduced very rapidly and the pyruvic acid formed will be the only hydrogen acceptor. This picture might serve as a simplified interpretation of the old phenomenon first observed by Pasteur (the so-called Pasteur effect), that in the presence of oxygen fermentation disappears or is depressed. The support for the hypothesis illustrated here is the observation of Lipmann (170) that the addition of large amounts of a very positive dye is able to regenerate the Pasteur effect in a system where this effect has disappeared.¹⁹

I wish, however, to point out that for several systems the illustration of respiration as a transfer of electrons through nucleotides and iron porphyrins is too simple.

As mentioned before in this review, small traces of succinic acid are necessary for the oxidation of pyruvic acid and glucose in animal tissue. Whether the succinic acid-fumaric acid system is interposed as an electron-transfer system between the alloxazine and iron porphyrin systems, as claimed by Szent-Györgyi, or whether it acts in another manner is a problem of great interest, also for the discussion of the Pasteur effect.

Tumor tissue (289), damaged tissue (289), and some tissue cultures (169) exhibit, in spite of a high respiration, a considerable aerobic glycolysis.

Colowick, Kalckar, and Cori (39) observed an aerobic glycolysis in kidney extract. The aerobic glycolysis (from glucose) disappears under anaerobic conditions, since glucose is not phosphorylated under these conditions. This aerobic "extract glycolysis" from glucose is probably due to an overproduction of hexose diphosphate (see section V), which by the action of glycolytic enzymes is converted into lactic acid. The authors discuss the possibility that the aerobic glycolysis in damaged cells is attributed to a too one-sided application of the oxidative energy on glucose phosphorylation which, in the presence of glycolytic enzymes, leads to lactic acid formation.

V. THE SIGNIFICANCE OF PHOSPHORYLATION IN OXIDATION-REDUCTION

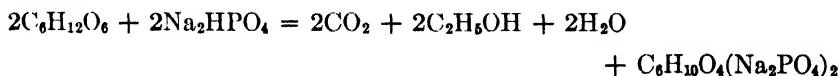
A. *The transfer of phosphate*

As pointed out in section IV, alcoholic and lactic acid fermentation can be described as a hydrogen transfer by "pyridine" from an aldotriose to the carbonyl group of acetaldehyde or pyruvic acid. One complication, however, has so far not been mentioned in this review (except in the equa-

¹⁹ Michaelis and Smythe (213) are of the opinion that the dyes inhibit primarily the phosphorylation of glucose, particularly the formation of hexose diphosphate.

tion of the dismutation of triose phosphate), that is, the problem dealing with the active form of the hydrogen donor. Whereas the hydrogen acceptor simply is acetaldehyde or pyruvic acid, the hydrogen donor in several cases is a much more complex system, the nature of which has not been revealed until recently; in fermentations the hydrogen donor is not triose but a phosphorylated triose.

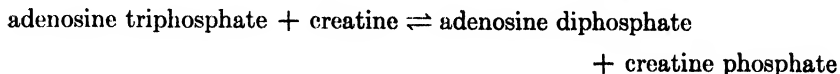
The importance of phosphate for the alcoholic fermentation was discovered in 1905 by Harden and Young (108, 109). These investigators proved that in cell-free fermentation of glucose (yeast juice), inorganic phosphate disappeared by an esterification with glucose; the accumulated ester was hexose-1,6-diphosphate. Moreover, they observed a close relationship between the phosphate esterified and the amounts of carbon dioxide and alcohol formed. One half of the sugar utilized was split into carbon dioxide and alcohol and the other half was esterified to hexose diphosphate. This relation is expressed in the so-called Harden-Young equation:



Later Robison (248) isolated hexose monophosphate from fermentation mixtures and showed it to consist of 60 per cent glucose-6-phosphate and 40 per cent fructose-6-phosphate. Usually dried autolyzed yeast accumulates much more hexose diphosphate than hexose monophosphate during fermentation, but investigations by Kluyver and Struyk (138) showed that the ratio between these two esters can readily be changed in favor of hexose monophosphate by dilution, and Smythe (260) showed that the yield of hexose monophosphate can be raised if certain redox dyes, for instance, rosinduline, are added. Addition of even very small amounts of arsenate (10^{-8} to 10^{-6} mole) entirely prevents the accumulation of hexose diphosphate and makes the fermentation independent of the addition of phosphate (110).

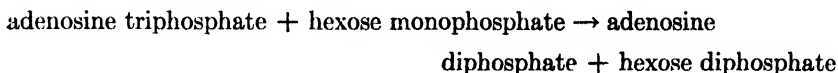
Meyerhof and collaborators (198) demonstrated the significance of the sugar phosphoric acid esters in the glycolysis in muscle tissue. Furthermore, Meyerhof and Lohmann and Parnas and Ostern and their coworkers have been able to demonstrate the mechanism of the phosphate transfer, a process which is closely connected with the fermentations in yeast and muscle tissue. Two phosphate esters are particularly important for the phosphate transfer in the metabolism of muscle tissue: adenylic acid, isolated in 1927 by Embden and Zimmermann (67), and creatine phosphate, discovered and isolated by Eggleton (62) and by Fiske and Subarow (85) in 1927-29. The function of these two esters has been revealed by the investigations of Lohmann, Parnas and Lundsgaard, and others.

Adenylpyrophosphate (adenosine triphosphate) is in enzymatic equilibrium with creatine according to the equation (181, 160):



Since the equilibrium constant is not far from 1, the free-energy change involved in this reaction is very small.

According to Euler and Adler (72) and to Ostern (233), the adenosine triphosphate is able to transfer one or two of its phosphate groups to sugar or to hexose monophosphate:



These processes cannot be reversed experimentally and give rise to a considerable liberation of free energy.

Furthermore, 2 moles of inorganic phosphate can be liberated from adenylpyrophosphate by an enzyme, adenylpyrophosphatase (117). For every mole of orthophosphate liberated from adenylpyrophosphate, 11,000 calories are set free as heat (ΔH) (198). Creatine phosphate is dephosphorylated only through the adenosine phosphate system, which can be considered as a phosphate-transfer system, since a minute amount of this nucleotide is able to dephosphorylate a large amount of creatine phosphate.

Adenosine triphosphate and creatine phosphate act exclusively as phosphate donors. Adenosine diphosphate can act both as phosphate donor and phosphate acceptor. Adenosine monophosphate (adenylic acid), hexose, and hexose monophosphate act as phosphate acceptors.²⁰ Adenosine monophosphate or diphosphate can be phosphorylated to triphosphate, not only by creatine phosphate but also by products formed in the fermentation of sugar. These phosphate donors,—phosphoglyceryl phosphate, phospho(enolic)pyruvate, and acetyl phosphate,—will be described in this section.

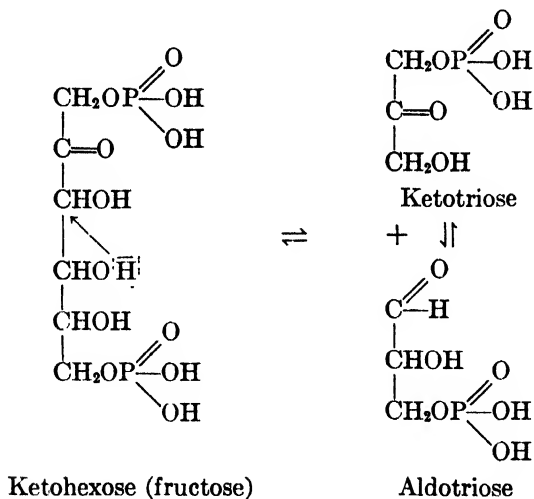
The classical work of Neuberg (224, 225) has been of very great importance for our understanding of fermentations as oxidation-reductions. The modern concept of the fermentation of phosphorylated sugars, however, was created by Embden and collaborators in 1933 (65) and developed by Meyerhof and coworkers (199). Owing to these brilliant investigations, every single step in the chain of reactions has been demonstrated and every single intermediate product has been isolated and identified. A further proof that the phosphate esters isolated are of physiological importance is the great rapidity with which these esters are converted into lactic acid

²⁰ Ostern *et al.* (234) have shown that in yeast systems adenosine (adenine-pentose) can be phosphorylated to the three different phosphorylation steps.

or into alcohol and carbon dioxide when small amounts of the right hydrogen and phosphate acceptors are present.

The mechanism of phosphate transfer connected with the fermentation has been cleared up by investigations of the Lemberg (23a) and the Cambridge schools (218). In a description of the Embden-Meyerhof scheme of fermentation it seems advisable to distinguish between three main phases: (1) Preparation of the actual hydrogen donor ("active sugar"), i.e., phosphorylation; (2) the oxidoreduction process proper; and (3) the regeneration of the hydrogen acceptor, i.e., anhydride formation and dephosphorylation.

1. *Phosphorylation of sugars.* In yeast, glucose (or fructose) is phosphorylated by adenosine triphosphate to hexose-6-phosphate and hexose-1,6-diphosphate (fructose diphosphate). In muscle tissue or tissue extracts, glycogen or starch is phosphorylated,²¹ but here also hexose diphosphate is formed. The hexose diphosphate then undergoes a cleavage²² by a reversible enzymatic reaction into two molecules of triose phosphate esters:



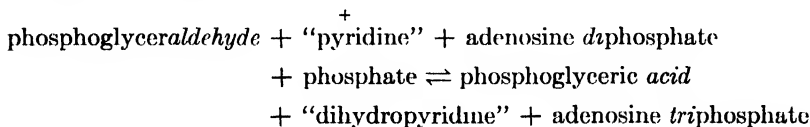
The enzyme which catalyzes this cleavage (or the reverse, the so-called aldol condensation) occurs in all tissues. This enzyme was called aldolase by Meyerhof and Lohmann (202, 203). The aldo-ester can be converted into the keto-ester or *vice versa* by a specific enzyme (isomerase).

2. *The oxidoreduction.* Until recently phosphoglyceraldehyde hydrate

²¹ The initial steps will be described in a later section.

²² Cf. the cleavage of rhamnose by some bacteria into propylene aldehyde and glyceraldehyde. The propylene aldehyde is immediately reduced to propylene glycol (137).

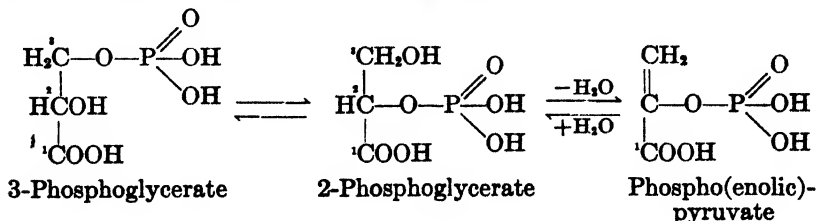
has been considered as the hydrogen donor proper, i.e., the substance which is dehydrogenated by the pyridine nucleotide; the latter then transfers the hydrogen to the carbonyl group of pyruvic acid or acetaldehyde. The end products of this oxidoreduction are phosphoglyceric acid and lactic acid or ethyl alcohol. This oxidoreduction, however, is not as simple as the scheme pictured in section III. Investigations from Needham's, Warburg's, and Meyerhof's laboratories (199) showed that simultaneously with the transfer of hydrogen from phosphotriose to the pyridine nucleotide, inorganic phosphate is taken up, yielding adenosine triphosphate. The primary acceptor of the inorganic phosphate was not known at that time, but it was shown that inorganic phosphate and adenosine diphosphate are necessary components in the oxidoreduction and that the inorganic phosphate is transferred to the adenosine diphosphate "by the energy of the oxido-reduction:"



The reversibility of this process will be referred to later in this review. The coupling between the hydrogen and the phosphate transfer in this system is compulsory. However, in the presence of even very small amounts of arsenate (10^{-5} mole) the oxidoreduction proceeds without uptake of phosphate; no uptake of arsenate was detectable.

No phosphorylation has been observed in connection with the transfer of hydrogen from "dihydropyridine" to the carbonyl groups of pyruvic acid or of acetaldehyde (206).

3. *The regeneration of the hydrogen acceptor.* Several steps are necessary for this regeneration: anhydride formation, dephosphorylation, deenolization, and (in the alcoholic fermentation) decarboxylation (225). Meyerhof and Lohmann have demonstrated every single step; all the steps are catalyzed by specific enzymes and most of the step reactions have been shown to be reversible. The oxidation product of triose phosphate, 3-phosphoglyceric acid, is converted by a reversible enzyme reaction into the 2-phosphoglyceric acid, in which the phosphate group is esterified to the hydroxyl group of the middle carbon atom. The 2-phosphoglyceric acid undergoes a dehydration yielding phospho(enolic)pyruvic acid:



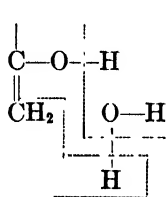
The phosphopyruvic acid has all the properties of an enolic ester (vinyl ester); i.e., it is hydrolyzed by small amounts of mercuric salts and by hypiodite (183).

The phosphopyruvic acid is dephosphorylated by adenosine monophosphate or diphosphate (238), yielding free pyruvic acid, mainly in the keto-form (carbonyl) and adenosine triphosphate:

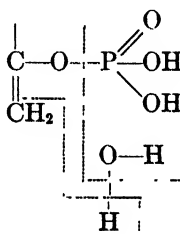
2 phospho(enolic)pyruvic acid + adenosine monophosphate

→ 2 pyruvic acid + adenosine triphosphate

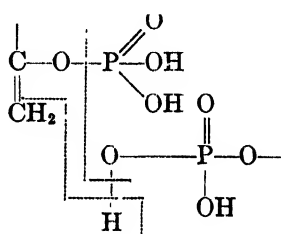
This reaction has so far not been demonstrated to be reversible. The ability of phospho(enolic)pyruvic acid to form pyrophosphate linkages must be ascribed to the simultaneous shift from an enolic to a keto structure:



(a) Conversion of enol-pyruvate to keto-pyruvate; $-\Delta F$ large.



(b) Conversion of phospho(enolic)-pyruvate to ketopyruvate + phosphate; $-\Delta F$ large.



(c) Formation of pyrophosphate linkage from phospho(enolic)-pyruvate; ΔF small.

B. The nature of the compulsory coupling between oxidoreduction and phosphorylation

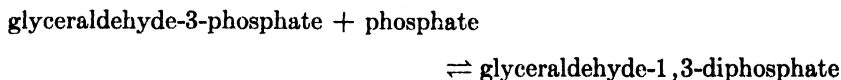
The most complex step in the glycolysis is the oxidoreduction between triose phosphate and pyridine nucleotide, because this step requires the uptake of inorganic phosphate and the presence of adenosine diphosphate as an acceptor of this inorganic phosphate. The nature of this compulsory coupling between triose phosphate oxidation and phosphate uptake have remained completely obscure.

Recently, investigations from Warburg's laboratory (220, 299) have revealed the nature of the compulsory coupling between oxidoreduction and phosphorylation. Since these discoveries are most fundamental and of the greatest consequence for our understanding of energetic couplings, much attention will be paid to them in this review.

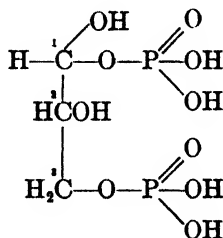
In 1939 Warburg and Christian (299) succeeded in the complete separation of different enzymes involved in the oxidoreduction of alcoholic

fermentation. This very high purification and separation of enzymes enabled Negelein and Brömel to observe and isolate a very important and interesting new ester, 1,3-diphosphoglyceric acid.

The following reversible reaction is supposed to take place:

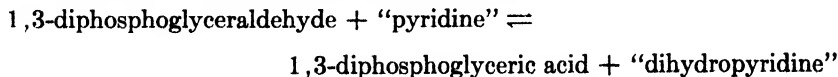


The new phosphate is linked to the aldehyde group²³ and the formula is as follows



i.e., the aldehyde phosphate replaces an aldehyde hydrate group. This diphosphotriose has never been isolated. If a small amount of Warburg and Christian's new crystalline enzyme is added to a solution of pyridine nucleotide and diphosphotriose, a rapid transfer of hydrogen from the triose to the "pyridine" takes place. The end products of this reaction are dihydropyridine nucleotide and diphosphoglyceric acid, which was isolated as the strychnine salt. The crystalline enzyme which catalyzes this oxidation is very active even in very small concentrations (0.8γ per cubic centimeter).

The oxidoreduction between diphosphotriose and pyridine is reversible:

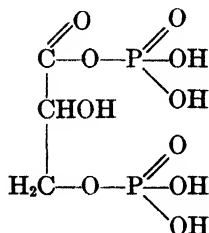


This is the oxidoreduction proper which is independent of inorganic phosphate and adenylic acid.

The aldehyde phosphate group of the diphosphotriose apparently represents the "active" sugar, since Warburg and Christian find that besides 1,3-diphosphotriose the 1-phosphotriose also is oxidized by the pyridine enzyme, although not nearly so rapidly; the well-known 3-phosphotriose is not oxidized.

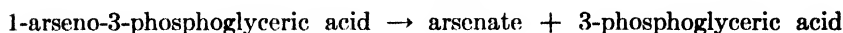
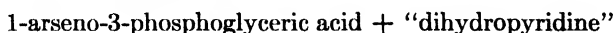
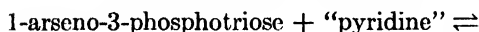
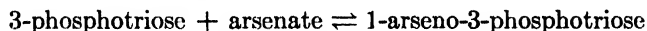
²³ Apparently non-enzymatic as, for instance, the carbonyl sulfite reaction (cf. 175).

The 1,3-diphosphoglyceric acid apparently has the following formula (Negelein and Bromel):



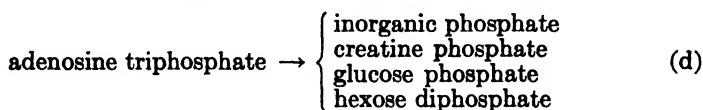
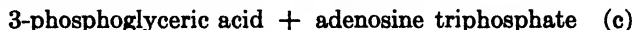
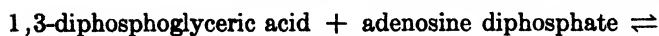
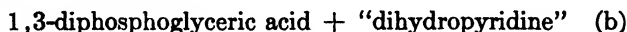
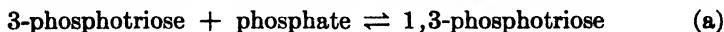
The phosphate group linked to the carboxyl group is labile, although no enzyme seems to catalyze the liberation of phosphate (mineralization) from this carboxyl phosphate. Just like phospho(enolic)pyruvic acid and creatine phosphate, the phosphate of the carboxyl phosphate is transferred to adenosine monophosphate or diphosphate by a specific enzyme. Whether the phosphate of the "glyceryl phosphate" first has to pass the pyridine nucleotide or dihydropyridine nucleotide before entering the adenine nucleotide is not known, but the pyridine nucleotide is definitely not involved in the primary uptake of inorganic phosphate. Addition of arsenate abolishes the compulsory coupling between oxidoreduction and phosphorylation.

Warburg suggests a series of step reactions. In the presence of arsenate the reactions are as follows:



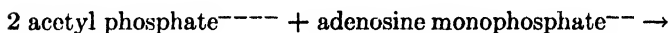
The last equation expresses the fact that the arsenate is liberated from carboxyl arsenate spontaneously and rapidly enough to replace phosphate in the oxidoreduction.

In case phosphate is taken up, the corresponding reactions are:



In Lebedew juice both phosphate groups contained in diphosphoglyceric acid are transferred *via* the adenine nucleotide to glucose, fructose, or hexose monophosphate. In living yeast one half of the phosphate is transferred to hexose; the other half is liberated (mineralized). The new C₃-diphosphates are also formed in muscle tissue; in this system the phosphates are transferred to creatine.

The isolation of a carboxyl phosphate able to phosphorylate adenine nucleotides has already led to a new fundamental observation. Lipmann (174), working with pyruvic acid dehydrogenase from lactic acid bacteria, observed that the oxidation of pyruvic acid to carbon dioxide and acetic acid requires inorganic phosphate or arsenate. No phosphate uptake was observable by the ordinary methods. The new interpretation of the phosphate uptake in the alcoholic fermentation led Lipmann (176) to suggest a formation of acetyl phosphate as the primary product of the enzymatic pyruvic acid oxidation. The formation of acetyl phosphate from pyruvic acid could not be demonstrated at that time, but Lipmann prepared acetyl phosphate synthetically and showed that in the presence of dried lactic acid bacteria phosphate is transferred from acetyl phosphate to adenylic acid according to the reaction:



The demonstration of this reaction is very important, because it again illustrates an extraordinary property of carboxyl phosphate,—the ability to phosphorylate the adenylic acid system. More recently Lipmann (177) has observed the formation of a very labile phosphoric ester in the bacterial oxidation of pyruvic acid. The properties of this labile ester correspond actually to those of acetyl phosphate.²⁴

As mentioned before, the pyrophosphate linkages in the adenine polyphosphates represent 11,000 calories (ΔH) which, besides carboxyl phosphates, only can be derived from guanidine phosphates and from phospho(enolic)pyruvic acid. The free energy of ordinary phosphoric esters (glycerophosphate, 6-phosphohexoses) can be calculated from rough estimations of equilibrium constants of hydrolysis of such esters. This calculation gives ΔF a value of about 1000 to 2000 calories.

The free energy of the aldehydehydrate-phosphate linkages can hardly exceed 1000 to 2000 calories, since the phosphorylation of triose in position 1 apparently takes place with inorganic phosphate (Warburg and Chris-

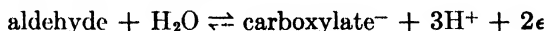
²⁴ Lipmann points out that the large liberation of energy in the hydrolysis of acetyl phosphate can also be used to acetylate compounds, for instance, choline. The name "phosphorylacetate" indicates that the ester can function as acetate donor (*cf.* 175).

tian). This means that an oxidation of an aldehyde-phosphate group to a carboxyl phosphate represents a conversion of a phosphoric ester with a potential energy around 1000 calories to a phosphoric ester having a potential energy of the order of magnitude of 10,000 calories.

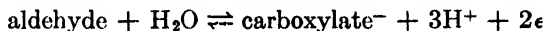
As a consequence of this great increase in the free energy (great $+\Delta F$) of the phosphate linkages in the conversion of an aldehyde-phosphate into a carboxyl phosphate, the fall in free energy of the *total* group is much smaller than the corresponding free-energy decrease between free aldehyde (+ water) and carboxyl groups. This again means that the normal potential of the system



is around 9 to 10,000 calories (=approximately 200 millivolts) higher than the normal potential of the system



An increase of 200 millivolts from the potential of the free aldehyde-carboxyl system will give a potential very near that of the pyridine system. The potential of



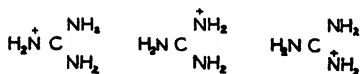
calculated from thermal data (Parks and Huffman (237), Borsook (26)) is 40 to 50 millivolts more negative than the hydrogen electrode, i.e., E'_0 (pH 7) = approximately -460 millivolts. This is also in agreement with some experiments of Green *et al.* (99), who found that glyceraldehyde in the presence of a specific enzyme (mutase) reduced benzyl viologen completely.

Warburg and Christian's recent observations show that the potential of the aldehyde-phosphate \rightleftharpoons carboxyl phosphate system amounts to nearly the same as the "dihydropyridine" \rightleftharpoons "pyridine" system (i.e., -250 to -300 millivolts). If the ratio of oxidant to reductant of the pyridine system is greater than 1, which probably is the case under physiological conditions, diphosphotriose will easily be oxidized by "pyridine."

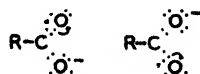
C. The relation between the electronic structure of phosphoric esters and their thermodynamic properties

Large free-energy decreases of reactions, as observed, for instance, in the hydrolysis of pyrophosphates, guanidine phosphates, carboxyl phosphates, and enolic phosphates, means that the stability of the products of reaction is much greater than that of the reactants. The modern physical and structural chemists lay stress on the phenomenon of resonance as a large factor in explaining the stability of molecular groupings. Molecules

like the guanidinium ion and carboxylate ion can be described as **resonating** between two or three symmetrical structures.



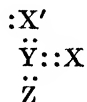
Guanidinium ion



Carboxylate ion

It is principally this resonance, stabilizing the guanidinium ion, which makes guanidine a base approximately 10^7 times stronger than ammonia, and this resonance preferentially stabilizing the carboxylate ion which makes carboxylic acids so much stronger than alcohols in acidity.

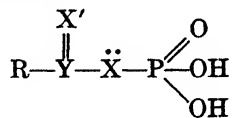
In general, molecular groups which have the following configuration exhibit high resonance:



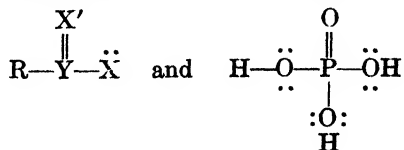
where the two X groups are the same or are closely alike in electron-attracting powers. Resonance is still more pronounced if Z is the same as X' with an unshared pair of electrons.

It is one of the fundamentals of thermodynamics that the maximal amount of free energy is liberated in a reaction converting a molecular group of particularly low stability (or high energy) to one of particularly high stability (or low energy). As we have seen, the carboxylate ion, guanidinium ion (also ions of monosubstituted guanidines like creatine), etc. represent resonating structures of particularly high stability. Carboxyl phosphates, guanidine phosphates, enolic phosphates, and pyrophosphates share the same thermodynamic characteristic: hydrolysis of the phosphate ester linkage liberates much more energy (five to ten times more) than hydrolysis of hydroxy phosphate esters.

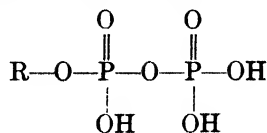
The phosphatic esters which are rich in energy actually display some common essential features in their structural composition. In all these esters we find the configuration:



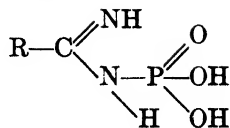
which can be hydrolyzed to give the configurations:



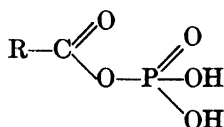
Ester linkages of high energy are as follows:



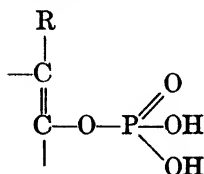
Organic pyrophosphate



Guanidino phosphate



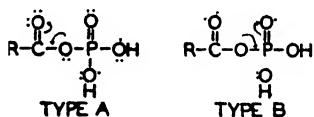
Carboxyl phosphate



Enolic phosphate

Only in the last compound, enolic phosphate, does X' differ from X, a difference which tends to decrease the resonance contribution to the stability of the ester and the hydrolysis product. In this case the hydrolysis product is stabilized by tautomeric shift to the more stable keto-form, pyruvic acid.

The configurations of all the phosphoric esters displaying high esterification energy, and only these, show one important characteristic feature which will be referred to as opposing resonance.²⁶ Since the phosphate molecule on the right exhibits resonance between the different hydroxyl or amino groups and the P=O linkage and groupings on the left also exhibit an analogous resonance, the bridge of the ester linkage (—O— or —N—) is influenced by opposing resonance between the organic group (carboxylate ion, guanidino ion) and the phosphate group, each making demands on the same atom for the independent resonating systems.



Resonance of type A therefore opposes that of type B, leading to less resonance energy for the groups combined as ester than for them when independent. The simultaneous elimination of two resonating structures makes carboxyl phosphates relatively very unstable. This instability of the ester in connection with the very high stability of the hydrolyzed product is responsible for the large liberation of free energy when this

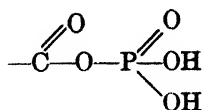
²⁶ Personal communication from C. D. Coryell.

kind of phosphoric ester is hydrolyzed. The great liberation of free energy by the hydrolysis of acetic acid anhydride is also attributed to opposed resonance of this type in connection with the high resonance of the two carboxylate ions. Negelein and Brömel (220) have investigated the ultraviolet spectrum of 1,3-diphosphoglyceric acid and have actually found a bond characteristic of the spectrum of acetic acid anhydride. Recently Lynen (190) found the same absorption band ($m\mu$ 217) for synthetically prepared acetyl phosphate. The phosphoric ester linkages with alcohol (hydroxyl) groups or aldehyde-hydrate groups (glycerophosphate, ordinary hexose phosphates, and monophosphotriose) do not exhibit opposing resonance and this, together with the lack of a resonating configuration of the free hydroxyl or aldehyde groups, will give a much smaller free-energy decrease upon the hydrolysis of this kind of phosphoric ester.

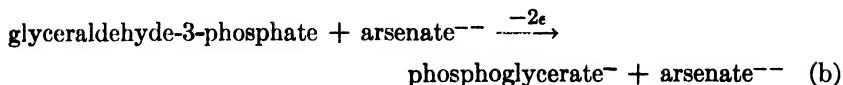
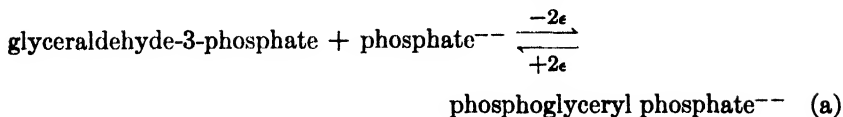
Warburg and Christian's separation of the enzymes involved in the biological oxidation of triose phosphate may also be of essential importance in obtaining values of the free energies of energy-rich phosphoric esters like carboxyl phosphate and adenylypyrophosphate. The possibilities of obtaining thermal data (heat of formation and heat capacity) for biologically important phosphoric esters are very small, because of the high requirements in purity and amount of substance. Thermal data for the inorganic phosphates exist but are very few and inaccurate. Latimer (159) estimates the pyrophosphate as "several tenths of a volt." More accurate thermal data of inorganic pyrophosphate would undoubtedly also be of interest in the study of adenylypyrophosphate. So far we know only the ΔH of the splitting of phosphate from adenylypyrophosphate. A large ΔF of such dephosphorylations makes, of course, direct equilibrium study impossible. These may, however, be applied to energy-poor phosphoric esters like carbonyl phosphates. Finally, free-energy changes can be measured by the potentiometric method which could, after the separation of all the "step enzymes" in the coupled oxidation of triose phosphate, be applied to the measurement of the free energy of some important phosphoric esters.

Assuming that the potentials of the systems of the triose phosphate oxidations are well defined, the following principles could be applied to obtain values for the free energy of the ester linkages in carboxyl phosphate and adenylypyrophosphate.

For the free energy of the ester linkage in

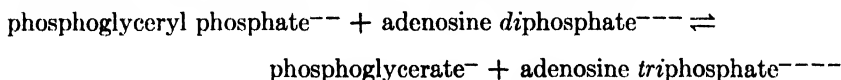


compare the redox potentials (ΔF) of the systems:²⁶

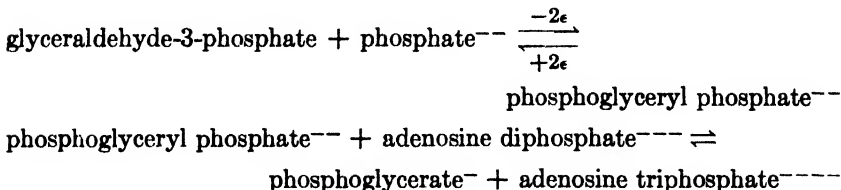


The difference between the redox potentials (E'_0 , pH 7) in the presence of phosphate and in the presence of arsenate would permit a calculation of the free energy of the glyceryl phosphate linkage. The difference between the two redox potentials might very well be about 250 to 300 millivolts.

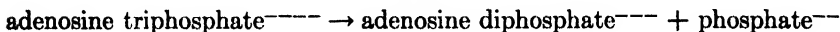
The free energy of the pyrophosphate linkages in adenosine triphosphate can be obtained from the free energy of the glyceryl phosphate ester linkage by correcting for the ΔF of the enzymatic reaction:



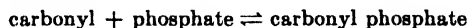
This ΔF , which is supposed to be small, could be obtained by direct equilibrium determinations. However, the free energy of the pyrophosphate linkages in adenylypyrophosphate might also be obtained directly by a potentiometric method according to the following principle: Suppose the normal potential of the total system:



is obtained and compared with the potential of the same system plus adenylypyrophosphatase (the enzyme which catalyzes dephosphorylation from adenosine triphosphate), giving rise to the extra reaction:



²⁶ The ΔF of the reaction



is not known, since the existence of carbonyl phosphate cannot be demonstrated by chemical methods. ΔF is probably very small (15 to 30 millivolts) and is of no importance for the present problem. Notice the appearance of a new acid equivalent in reaction b.

then the difference between the two potentials would give the ΔF of the dephosphorylation of one pyrophosphate linkage in the adenosine triphosphate. Assuming that the ΔF of the adenylypyrophosphate dephosphorylation is of the same order of magnitude as the ΔH , i.e., approximately 10,000 calories per mole of phosphate, then the redox potential of the system which oxidizes phosphotriose to phosphoglycerate and phosphorylates adenosine diphosphate to adenosine triphosphate should drop approximately 250 millivolts when adenylyphosphatase²⁷ is added. The theory developed here is also illustrated in figure 2 of section X.

The clarification of the coupling between the triose phosphate oxidation and the uptake of inorganic phosphate represents one of the greatest advances in modern biology. The nature of the energetic coupling of living systems has always been wrapped in a shroud of mystery. For the first time since this recent discovery of the Warburg school, a complete description of a biological coupling is possible. By following this new line a clarification of other energetic couplings is to be expected.

D. Coupling between respiration and phosphorylation

A coupling between respiration and phosphorylation has been observed in hemolyzates of red blood corpuscles. Lennerstrand and Runnstrom (1965) observed such a coupling in dry yeast preparations and found that triose phosphate was oxidized to phosphoglyceric acid.

Furthermore, phosphorylations coupled to respiration have been observed in animal tissue. This was first shown in minced kidney cortex and extract from kidney cortex (119). Such a system shaken with oxygen shows an intensive respiration. If phosphate and glucose are present and fluoride is added in order to stop dephosphorylation, most or all of the inorganic phosphate disappears in $\frac{1}{2}$ to 1 hr. The phosphoric ester which accumulates is mainly fructose-1,6-diphosphate. Fructose-6-phosphate added is phosphorylated to fructose-1,6-diphosphate. A new kind of esterification, so far only observed in kidney cortex, is the rapid phosphorylation of glycerol in kidney extracts; the ester formed was identified as the levorotatory α -glycerophosphate (123). Adenylic acid is phosphorylated rapidly to adenylypyrophosphate, an observation which corresponds to that of Engelhardt (68) and of Dische (60), made in experiments with red cells. The coupled phosphorylation and oxidation of fumaric or malic acid to phosphopyruvic acid has been mentioned and discussed in a previous section in this review.

All these phosphorylations depend on the rate of respiration; an inhibition of the respiration by cyanide gives an equal decrease in the phos-

²⁷ Only that fraction of the enzyme which splits one phosphate from the adenosine triphosphate. Such an enzyme can very easily be obtained from lobster muscles.

phorylation. Phloridzin inhibits the phosphorylation much more than the respiration.

Addition of 5 to 10 mg. of citric acid, glutamic acid, or another dicarboxylic acid, in some cases also alanine, approximately doubles the respiration, and the increase in phosphorylation of sugars, glycerol, or adenylic acid may be even larger.

Recent experiments of Colowick, Welch, and Cori (40) with dialyzed extracts show that the coupled phosphorylation system needs the following components: magnesium ion, succinic acid (about 0.1 mg. per cubic centimeter is sufficient), adenylic acid ($M/1000$ is a sufficient concentration), and pyridine nucleotide (cozymase). These experiments showed furthermore that extracts which had been dialyzed and aged for 24 hr. at 5°C., and therefore had oxidized succinic acid only one level to fumaric acid, still were able to phosphorylate glucose. Recent investigations of Belitzer and Tsibakova (18) on minced heart muscle also indicate that the step succinate $\xrightarrow{-2e}$ fumarate can give rise to phosphorylation.

These observations are of importance, because the succinic acid-fumaric acid system is quite different from the other systems known to be coupled with phosphorylations. The succinic acid oxidation is a desaturation, like the oxidation of saturated fatty acids. How the phosphate can enter such a system is not yet understood; perhaps an uptake of phosphate during the oxidation yields phosphomalic acid instead of fumaric acid.

Succinic acid dehydrogenase from kidney seems, however, not to be dependent on phosphate, since an intensive oxidation occurs even at very low phosphate concentrations ($M/3000$). Whether the succinic acid is oxidized directly by cytochrome *c* or whether the dehydrogenase possesses a prosthetic group (for instance, a flavin nucleotide) is so far an unsolved problem. The normal potential of the succinate-fumarate system, E'_0 (pH 7), is approximately 0; the normal potential of cytochrome *c* is +270. This great potential difference between the two systems is probably of importance for the ability of the succinate oxidation to cause phosphorylation.

Furthermore, Colowick, Welch, and Cori (41) observed that if glucose is added to an extract (in the absence of fluoride) it is not oxidized; however, in the presence of 0.2 mg. of succinic acid a very intense oxidation takes place. This observation corresponds to that of Annau and Erdős (4) and of Banga, Ochoa, and Peters (8) that pyruvic acid only is oxidized in the presence of succinic acid.

Recent experiments by Colowick, Kalckar, and Cori (39) show a quantitative relation between the combustion of glucose (complete oxidation to carbon dioxide) and phosphorylation. Cell-free, dialyzed extracts of heart muscle are occasionally able to oxidize glucose completely. For every mole of glucose oxidized to carbon dioxide, an additional 5 to 6 moles of

glucose disappear, 5 moles of which are accumulated as fructose diphosphate. These quantitative relationships indicate strongly that at least ten, if not all twelve, steps in the glucose combustion can give rise to phosphate uptake.

Experiments with kidney and heart extracts show that the oxidation of citric acid and glutamic acid can give considerably more phosphorylation than oxidation of succinate.

In some experiments with heart extract, the oxidation of pyruvate (but not of succinate) gave remarkably high yields of phosphorylation. Per millimole of oxygen consumed, 4 millimoles of phosphate were taken up. Belitzer and Tsibakova and Ochoa have observed just as high ratios of P/O_2 or even higher, and these authors also point out that the high ratio P/O_2 may be explained by an additional uptake of phosphate when the hydrogen passes from one hydrogen-transfer system to another. Belitzer and Tsibakova's experiments exclude any anaerobic dismutations as a source of phosphorylation. The assumption that a transfer of hydrogen from one transfer system to the next (for instance, from the pyridine to the alloxazine nucleotide) is able to provide energy for phosphorylations deserves attention. There is no reason to assume that the step metabolite \rightarrow "pyridine" (or "thiamine") is the only step in the long chain of hydrogen transfer which can provide energy for phosphorylations.

The large accumulation of hexose diphosphate in heart extracts, even in the absence of fluoride, when glucose or pyruvate are oxidized is presumably attributed to the lack of phosphatases.

This accumulation of hexose diphosphate in cell-free heart muscle extracts which burn glucose is actually the analog to the Harden-Young reaction in cell-free yeast juice. Hexose diphosphate also is accumulated in kidney extracts, which oxidizes glucose and pyruvate to carbon dioxide. The accumulation is, however, much smaller than in heart extracts and the main part of the glucose which disappears in addition to the combustion is converted into lactic acid. The lactic acid formation is not able to phosphorylate extra glucose.

The lactic acid formation from glucose in kidney extracts is therefore, at least in most cases, an exclusively aerobic phenomenon. The ratio moles of glucose utilized/moles of glucose oxidized is frequently much less than 6, presumably because kidney extracts contain large amounts of adenylypyrophosphatase.

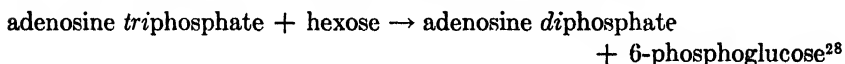
In the living cell this ratio is also much smaller than 6, since only a small fraction of the phosphate taken up in the oxidation of metabolites needs to be transferred to glucose²⁸ for the autocatalysis of the respiration; the main part is presumably connected with the specific cellular structures.

²⁸ Since the hexokinase was separated from a conversion enzyme, i.e., the enzyme which catalyzes the conversion of 1-phosphoglucose to 6-phosphoglucose (see section VIII), the primary sugar ester formed is 6-phosphoglucose.

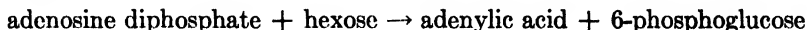
The formation of hexose diphosphate or of ordinary hexose monophosphate (6-phosphoglucose) from adenylypyrophosphate (polyphosphorylated by oxidation) and glucose or fructose represents a considerable fall in free energy, since a pyrophosphate linkage contains more than 10,000 calories, whereas alcohol-phosphate linkages represent hardly more than 1000 calories. It is likely, however, that in the living cell the energy of the pyrophosphate is utilized to a much greater extent. 1-Phosphoglucose (Cori ester, *cf.* section VIII, D), the precursor of polysaccharides, is probably formed from adenylypyrophosphate.

The yeast enzyme, hexokinase, which was discovered by Meyerhof in 1927, catalyzes the transfer of phosphate from adenylypyrophosphate to glucose or fructose. This enzyme system has recently been studied by the author in collaboration with Colowick (Colowick and Kalckar, 1940-41; experiments to be published).

These studies show that the enzyme (kept as an ammonium sulfate precipitate) in the presence of magnesium ions, adenosine triphosphate, and hexoses (glucose or fructose), catalyzes the reaction:



The reaction, which proceeds with considerable rapidity, can be followed not only by chemical methods but also manometrically, since one acid equivalent is liberated when an alcohol phosphate replaces a pyrophosphate. In this system only one pyrophosphate linkage is split; however, the remaining pyrophosphate is utilized (for hexose phosphorylation) if a heat-stable protein isolated from muscle tissue is added to the enzyme system. In the presence of hexokinase, magnesium ions, and the heat-stable protein, adenosine diphosphate is able to react with hexose according to the equation:



The heat-stable protein which, in addition to the yeast enzyme, is necessary for the last-mentioned reaction, occurs in muscle extracts but neither in liver nor in kidney extracts. The active protein is precipitated by ammonium sulfate (and can be purified by fractionation), by sodium sulfate, and by trichloroacetic acid. The trichloroacetic acid precipitate is soluble in alkali, and the solution exhibits full activity. The protein is inactivated when kept in alkaline solution but is completely reactivated by reduced glutathione or cysteine. The activity of the protein is not decreased by 15 min. boiling in 0.1 *N* hydrochloric acid. Pepsin hydrolyzes the protein and the activity disappears.

The heat-stable protein is active in very small amounts; 1 γ of purified

protein per cubic centimeter still exhibits considerable activity. Insulin is inactive in the enzyme test.

The function of the protein in the phosphate transfer from the organic pyrophosphate compound to hexose is not yet known. A potassium chloride-bicarbonate solution, used for the extraction of myosin, extracts several times more of the active protein than is obtained by an ordinary water extraction. This observation, together with the fact that the protein is absent in liver and kidney extracts, deserves attention (cf. section IX, D).

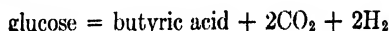
The distribution of the phosphate energy will undoubtedly be one of the major problems in the future.

VI. THE SYNTHESIS OF FATTY ACIDS FROM SUGARS

Little is known about the pathway of fatty acid formation from sugar. However, a good deal can be learned by studying some bacterial fermentations where fatty acids are formed. The best known of this kind of fermentation is the butyric acid fermentation.

A. The butyric acid and butanol fermentations

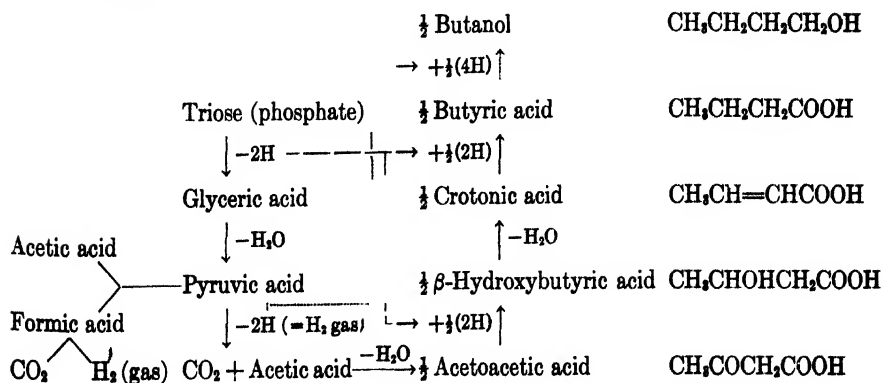
The anaerobic spore-form *Clostridium butyricum* ferments sugar according to the equation:



Besides these products, a varying amount of acetic acid is formed.

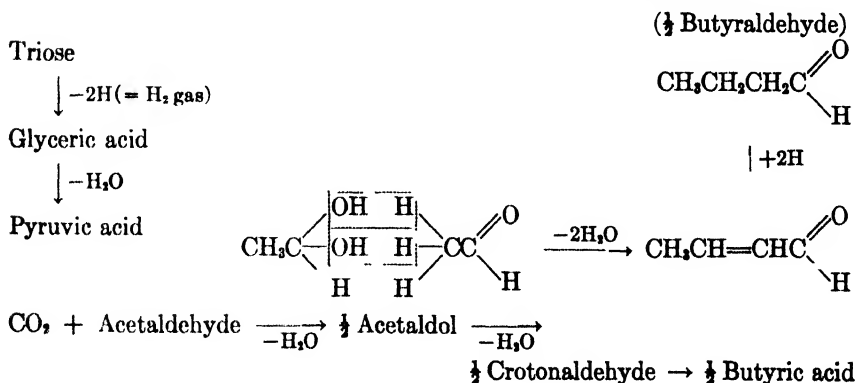
If butyl alcohol (butanol) is formed, no hydrogen gas arises, since the formation of butanol is equivalent to equal quantities of butyric acid and hydrogen gas.

The majority of investigations have been carried out on the fast growing *Clostridium acetobutyricum* (Fernbach). On the basis of these investigations, a reasonable scheme of the butyric acid fermentation would be as follows:



Most of the hydrogen gas presumably arises in the oxidation of pyruvic acid to acetic acid and carbon dioxide, because of the very low potential of this system. The condensation of acetic acid to acetoacetic acid has been demonstrated (247).^{28a} Addition of acetic acid to a butanol fermentation mixture yields a quantitative amount of acetone but no butanol (118). This is due to the fact that all the hydrogen formed is used in the butanol fermentation; acetoacetic acid formed from the added acetic acid therefore accumulates and is spontaneously decarboxylated. The fact that added acetic acid or acetoacetic acid is not converted to butyric acid or butanol can therefore not be used as an objection against the theory of intermediate formation of acetoacetic acid in these fermentations. The reduction of acetoacetic acid to β -hydroxybutyric acid is a well-known reaction in animal tissues (96, 115). The reduction of crotonic acid to butyric acid has been demonstrated by Bernhauer (21).

Another group of investigators lays more stress on the condensation of acetaldehyde to acetaldol as the source of butyric acid. This theory is supported by two observations. Fiachn's (103, 104) experiments with the mould *Endomyces vernalis* show that crotonaldehyde, the anhydride of acetaldol, is converted to fats. Kuhn and collaborators (155) have prepared stearic acid synthetically from crotonaldehyde. These two observations suggest that crotonaldehyde may be an essential intermediate, not only in the formation of palmitic acid but also in the butyric acid fermentation. The following scheme illustrates the crotonaldehyde theory:



It is not easy to say which of the two schemes best illustrates the butyric acid fermentation. The formation of acetoacetic acid and the reduction of this substance to β -hydroxybutyric acid are reactions which have been

^{28a} A direct formation of acetoacetic acid from acetic acid is unlikely, because of the high stability of the carboxyl group. The acetoacetic acid is perhaps formed from acetyl phosphate or from acetylpyruvic acid (cf. Krebs).

experimentally demonstrated in animal tissue. The formation of acetaldehyde from acetaldehyde has also been demonstrated, but the removal of the second molecule of water, yielding crotonaldehyde, is so far purely hypothetical. The addition of crotonaldehyde, on the other hand, gives rise to an increased formation of higher fatty acids. Feulgen's discovery of palmital (80), the aldehyde of palmitic acid, is of considerable interest in this discussion.

A reduction of crotonaldehyde to butyraldehyde is thermodynamically more likely than a reduction of butyric acid to butyraldehyde. Butyric acid, on the other hand, actually accumulates in the first phase of a butanol fermentation. If it is assumed that crotonaldehyde is reduced to butyraldehyde, an equilibrium between crotonaldehyde and butyric acid (far to the side of the latter substance) has to be postulated. Both schemes, however, illustrate very clearly the characteristic feature of a mixed assimilatory and dissimilatory fermentation: (1) The hydrogen acceptors are represented by the anhydrides of the first oxidation level (crotonaldehyde) or second oxidation level (acetoacetic acid), respectively, and, furthermore, by the anhydride of one of the reduction products (crotonic acid). (2) After a "concentration" of the oxygen at one end of the molecule (transformation of glyceric acid to pyruvic acid), one carbon is sacrificed as carbon dioxide and the other part (2C) is used for condensation.

The second scheme of butyric acid fermentation actually illustrates a sort of coupling between decarboxylation and a condensation, leading to fatty acid formation.

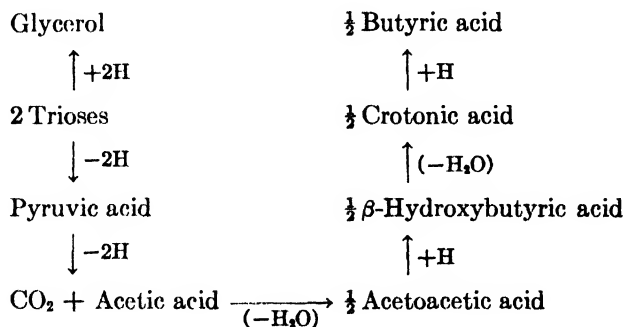
Fatty acid formation from sugar, regardless of whether it occurs in microorganisms or in animal tissues, is always connected with decarboxylation, simple or oxidative. Thiamin (vitamin B₁) is known to be of importance in the transformation of sugar into fatty acids (194). Transformation of glycerol into fatty acids gives rise to less carbon dioxide than the transformation of sugars to fatty acids. The most extreme illustration of this fact is van Niel's demonstration of the quantitative transformation of glycerol into propionic acid by propionic acid bacteria. This phenomenon has already been discussed.

The pathway of fatty acid formation from sugars in animal tissue is not known. The different possibilities have been discussed in a comprehensive review by Smedley McLean (259). The recent work of Schoenheimer and Rittenberg (256), using deuterium or radioactive carbon as tracers, will undoubtedly be able to throw considerable light on the pathway of fat formation in the animal organism.

Probably fatty acid formation from sugar in animal tissues is a mixed respiration and fermentation process. Since hydrogen gas is never formed

in animal tissues, this transformation can hardly be due to fermentation only.

If the triose is dismuted into glycerol and glyceric acid, sugar can be transformed into fatty acids by a pure fermentation without evolution of hydrogen.



The simultaneous formation of glycerol might be of importance for the formation of glycerides (fats).

B. The formation of higher fatty acids

The bacterial fermentative transformation of sugar into fatty acids, even the formation of caproic acid from alcohol (11), represents sources of energy for the growth of bacteria.

There is reason to believe that the transformation of sugar into fatty acid and carbon dioxide, occurring on a large scale in several animals, also represents a source of energy available for endergonic processes.

The understanding of the mechanism of fatty acid formation from sugar is closely connected with the knowledge of the oxidation of fatty acids. Knoop's hypothesis of β -oxidation has been of essential importance for the understanding of fatty acid oxidation. In this connection it is of interest that acetic acid condensation leads to a β -keto acid. According to the classical experiments of Knoop (139), every mole of fatty acid gives 1 mole of acetoacetic acid. Recent experiments (245, 23) show that in several cases more than 1 mole of acetoacetic acid can be formed from a molecule of the higher fatty acids, but these new observations are not in disagreement with the concept of β -oxidation.

Until recently the general opinion was that fatty acid dehydrogenases are strictly dependent on the integrity of the cell. This is not the case, since Leloir and Murry (163) and later Welch and Cori (301) found that homogenized liver tissue oxidizes butyric acid; the latter authors found a large increase in the oxygen consumption after addition of butyric acid to homogenized liver tissue. Furthermore, Konrad Land and collaborators

(143, 144) have recently been able to demonstrate a palmitic acid dehydrogenase in liver extract, which in the presence of methylene blue oxidizes palmitic acid to oleic acid. Adenylic acid (a pure product from Ostern's laboratory was used) is a necessary component of the palmitic acid dehydrogenase. These findings are of importance for the understanding of the nature of fatty acid dehydrogenases. The first step of fatty acid oxidation (desaturation) thermodynamically belongs to the hydrocarbon \rightleftharpoons ethylene type, like succinic acid \rightleftharpoons fumaric acid.

Schmidt (255) finds that the respiration in dialyzed heart muscle is increased considerably by the addition of phospholipids (purified).

The nature of fat formation, i.e., esters of glycerol (and phosphate) and carboxyl groups of fatty acids, is not known. The fundamental discovery of fatty aldehydes like palmital and their esterification with glycerophosphate (Feulgen (80)) may give the solution of the problem of fat formation. Oxidation of palmital-glycerol ester would yield a palmitin glyceride.

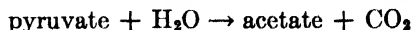
VII. THE SYNTHESIS OF NITROGEN COMPOUNDS

A. Assimilation of nitrogen

A few words about nitrogen fixation might be useful in this review.

The bacterial nitrogen fixation was discovered by Winogradsky in 1899 (305). Winogradsky was also the first to realize that the nitrogen fixation was a reduction of $N\equiv N$ to ammonia or derivatives of ammonia. As pointed out by Burk and Horner (33), however, a reliable demonstration of ammonia formed from nitrogen is difficult, because the process is so slow that it is difficult to distinguish it from an autolysis of the bacteria (azobacteria). Bortels in 1931 (28) discovered that the reduction of nitrogen to ammonia is catalyzed by molybdenum (*cf.* the Haber process); how the molybdenum acts, as an electron-transfer system or in some other manner, has not yet been ascertained.

In contrast to the reduction of the double bond in oxygen ($O=O$), the reduction of the triple bond in nitrogen ($N\equiv N$) is an endergonic reaction (*cf.* Lewis (166)). As pointed out by Burk, the stable $N\equiv N$ bond can be reduced only by hydrogen. It is not unlikely that the oxidation of pyruvic acid, because of the great negativity of the system

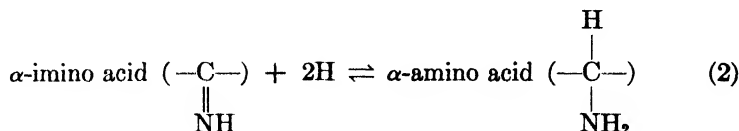
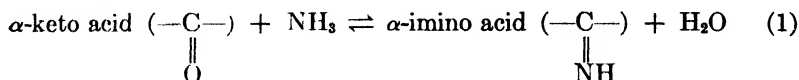


is an important energy source for the reduction of nitrogen.

B. The formation of amino acids from sugars and related compounds

In 1925 Knoop and Oesterlin (141) showed that palladium and hydrogen can reduce keto acids and ammonia to amino acids. Neubauer (223), Embden, and others demonstrated the deamination of amino acids to the

corresponding keto acids Knoop and Oesterlin in model experiments reduced α -keto acids with palladium and hydrogen in the presence of ammonium salts and obtained a high yield of the corresponding amino acids. They interpreted the reaction as follows.



Thus the imino acid replaces the keto acid as hydrogen acceptor; the labile nitrogen in the imino acid is "fixed" by the reduction to amino acid. That the oxidative deamination and the reductive amination are reactions of importance in living systems was established in 1937-38 by two discoveries, (1) Warburg and Christian's purification of the *d*-amino oxidase, and (2) Adler and Euler's demonstration of the enzymatic reduction of α -ketoglutaric acid and ammonia to glutamic acid.

1. *The d-amino oxidase* was first described by Krebs (146), who observed that a long series of *d*-amino acids (the optically active amino acids in proteins are mainly *l*-amino acids) are oxidized if added to water extracts of kidney cortex or liver tissue. Warburg and Christian (298) purified Krebs' oxidase and separated it into a protein and a nucleotide which was identified as a flavin-adenine nucleotide (*cf.* section IV). The alloxazine ring of the nucleotide accepts the hydrogen from the amino group, and the hydroalloxazine formed gives the hydrogen to oxygen, which is reduced to hydrogen peroxide. Negelein and Brömel (219) have shown that the hydrogen peroxide in the pure enzyme preparations free from catalase oxidizes the pyruvic acid formed from alanine to acetic acid and carbon dioxide. Proline and valine also are oxidized by the pure *d*-amino oxidase.

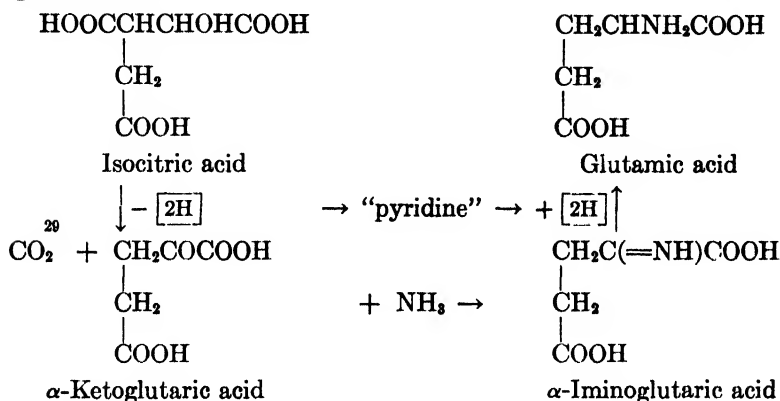
2. *The glutamic acid-ketoglutaric acid system.* Adler (1, 2), in Euler's laboratory, demonstrated that Knoop and Oesterlin's reductions of keto acids in the presence of ammonia to amino acids also occur in biological systems. Euler and coworkers (73) showed that glutamic acid is oxidized by a pyridine nucleotide (Warburg's coenzyme) to α -ketoglutaric acid and ammonia.

Adler prepared the hypopyridine nucleotide in large amounts and succeeded, in the presence of the specific glutamic acid dehydrogenase, in reducing α -ketoglutaric acid and ammonia to glutamic acid.

If the pyridine nucleotide is kept reduced by a hydrogen-donor system (e.g., alcohol-aldehyde + specific protein enzyme), catalytic amounts of

pyridine nucleotides are able to reduce large amounts of ketoglutaric acid and ammonia to glutamic acid.

Adler, Euler, Günther, and Plass (3) recently, in a very interesting paper, succeeded in converting citric acid (or isocitric acid) quantitatively to glutamic acid:

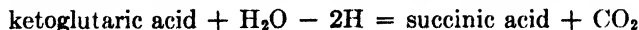


The conversion of isocitric acid and ammonia into glutamic acid is actually a "fermentation." The isocitric acid is formed from citric acid through the anhydride *cis*-aconitic acid (191, 192).

The position of citric acid in metabolism is not clear. A citric acid dehydrogenase (better "isocitric acid dehydrogenase") was first demonstrated by Thunberg. According to Krebs and Johnson (151), citric acid is formed by a condensation between oxaloacetic acid and an oxidation product of sugar, presumably acetic acid.

Simola and collaborators (258, 125) recently have been able to demonstrate extensive formation of citric acid in animal tissue *in vivo* as well as *in vitro* when pyruvic and malic acids were added. This might be due to a formation of acetic acid and oxaloacetic acid which immediately condense. It is not unlikely that the formation of citric acid is connected with the complex oxidation of acetic acid.

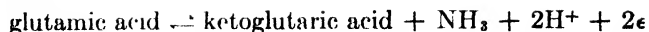
Krebs and Cohen (150) have recently shown an interesting dismutation of α -ketoglutaric acid and ammonia into glutamic acid, succinic acid, and carbon dioxide. In the absence of ammonia the dismutation proceeds to a much smaller extent. Apparently the iminoglutaric acid is a better hydrogen acceptor than the ketoglutaric acid is (*cf.* Krebs). The step



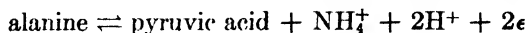
corresponds to the oxidative decarboxylation of pyruvic acid to acetic acid and carbon dioxide.

²⁵¹Spontaneous decarboxylation (*cf.* acetoacetic acid).

The system



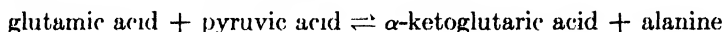
is a reversible redox system with an E'_0 (pH 7) = -0.50 millivolt (Borsook (26)). Wurmser and Fillitti-Wurmser (310) found that the system



is also a reversible redox system with an E'_0 = -0.48 volt. A reductive formation of alanine from pyruvic acid and ammonia is thus possible. Glycolysis in the presence of ammonia would therefore yield alanine instead of lactic acid.

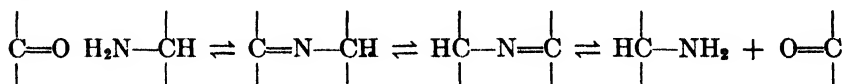
C. Transamination

Braunstein and Kritzman (31) made the important discovery that ketodicarboxylic acids and α -amino acids or *vice versa* react in the presence of specific enzymes (aminopherases) in such a manner that the amino group is transferred to the keto group, for instance:



One of the reacting components, either the keto acid or the amino acid, has to contain two acid groups as, for instance, glutamic acid or aspartic acid (the corresponding keto acids, phosphoserine and homocysteinic acid, are also active (30)).

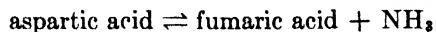
Braunstein and Kritzman interpret the transamination as a condensation, forming a Schiff base:



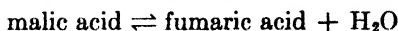
Only *l*-amino acids are transaminated. According to Braunstein, the transamination is the only way in which *l*-amino acids are oxidized. The Braunstein enzyme aminopherase is found in large amounts in all animal tissue; the glutamic acid represents the most important amino-transfer system.

D. The aspartase system

Quastel and Woolf (246) and Virtanen and Tornanen (286), working with *B. coli*, demonstrated a new type of amination-deamination:



The reaction is related to the equilibrium



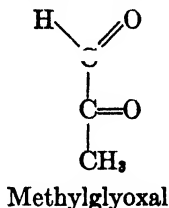
The ΔF values for these equilibrium reactions are less than 1000 calories.

Possibly deaminations related to the aspartase reaction are responsible for the reduction of amino acids to fatty acids in certain strains of *Clostridia* (Stickland). The unsaturated compound formed by deamination is the hydrogen acceptor proper.

E. The formation of peptides from amino acids

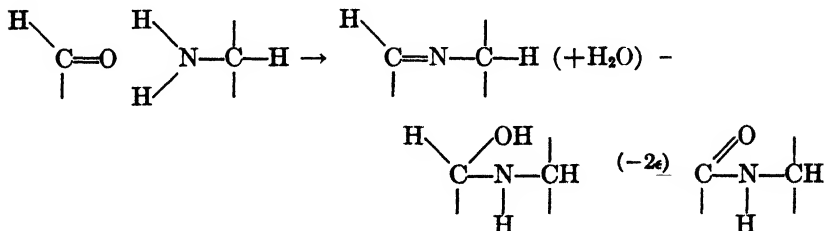
Peptides can be formed directly from amino acids if the latter are present in very high concentrations. The equilibrium lies far to the side of hydrolysis (*cf.* phosphoric esters in the presence of phosphatases). There is reason to believe that peptide formation in tissues is always coupled with oxidoreduction just like phosphorylations.

Linderström-Lang (168) points out that a primary reaction between a carbonyl group and an amino group followed by an oxidation of this linkage yields a peptide bond. He calls attention to the fact that, in order to obtain a typical polypeptide of α -amino acids, the amino group has to react with a dicarbonyl compound like methylglyoxal, where reductive amination of the 2-keto groups makes complete the addition of a new amino acid residue to the polypeptide chain



Methylglyoxal was discovered by Neuberg (226) in toluene-treated glucose-fermenting yeast. Methylglyoxal is formed by non-enzymatic dephosphorylation of triose phosphate (202).

The oxidation of a carbonyl-amino compound to a peptide shows some resemblance to an oxidation of an aldehyde (*cf.* also xanthine oxidation):



It is of importance to notice that both the condensation and the oxidation are exergonic reactions, indicating that peptide linkages are rather stable structures. The stability of the peptide structure able to resonate between

the amide and the enolic configurations is also pointed out by Pauling and Niemann (242).

The author of this review is inclined to think that carboxyl esters, like carboxyl phosphates (which yield pyrophosphate), glycerides (fats), and peptides are formed from the corresponding carbonyl esters by oxidation. This mechanism has been established in the case of carboxyl phosphate formation (Warburg, Negelein, Lipmann), is likely in the case of glycerides (Feulgen), and it may very well be that the "potential" energy of the carbonyl group is the driving force also in the peptide formation.

VIII. THE SYNTHESIS OF MONO- AND POLY-HEXOSES

A. *Photosynthesis*

In the green plant, sugars are formed from carbon dioxide and water with light as the energy source. Carbon dioxide is the hydrogen acceptor and water the hydrogen donor. Since the potential difference between the hydrogen donor and the hydrogen acceptor is 1200 to 1300 millivolts (= 56,000 calories) to the wrong side, the hydrogen donor belonging to the system of high potential and the hydrogen acceptor to the system of low potential, a supply of at least 56,000 calories per mole from an outside source of energy is necessary. This energy source is the light.

In the field of photosynthesis three main lines have been followed: (1) The number of quanta absorbed per mole of oxygen developed from water (the so-called quantum yield); (2) the constitution and mode of action of chlorophyll; (3) the intermediate reactions in the formation of sugar from carbon dioxide and of oxygen from water.

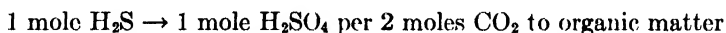
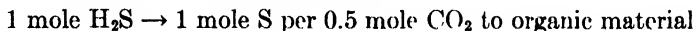
The chlorophyll of green plants was isolated by Willstätter and Stoll in 1910-12; these two investigators worked out the constitution of chlorophyll. Chlorophyll is a porphyrin compound in combination with magnesium. The chlorophyll of the photosynthetic active bacteria is very closely related to that of the green plants. Investigations of Hans Fischer *et al.* showed that the vinyl group, $\text{CH}_2=\text{CH}-$, in plant chlorophyll is replaced by an acetyl group, $\text{CH}_3\text{CO}-$, in bacterial chlorophyll. We have, however, only very limited knowledge about the mode of operation of chlorophyll, mainly because photosynthesis so far cannot be separated from the cell structure.

It seems certain that chlorophyll and not the carotinoids is involved directly in the light reaction, i.e., in the absorption of light. It has been suggested that chlorophyll transfers hydrogen, an assumption which seems reasonable but so far has no experimental basis.

The third problem, the pathway of sugar formation from carbon dioxide and water, has until recently not been treated experimentally, only specu-

latively. The work of van Niel, Gaffron, and others with the photosynthetic purple bacteria and the work of Ruben, Perlmann, and collaborators with radioactive carbon isotopes have given us the basis for an understanding of the reaction.

van Niel (228) found that the bacterial photosynthesis in some respects differs from that of the green plants: (1) no oxygen is formed; (2) the bacteria can live and grow without oxygen, provided light is present; (3) the bacteria can grow in the dark, provided oxygen is present; (4) several of the bacteria require hydrogen sulfide as well as carbon dioxide. van Niel found the following equations:



Does hydrogen sulfide replace water or does it replace ordinary metabolites? A decisive answer to this question was given by Gaffron's demonstration of a bacterial photosynthesis without hydrogen sulfide. He (89) found that in some purple bacteria photosynthesis of hydrogen sulfide takes place in the absence of hydrogen sulfide; in these cases fatty acids replace hydrogen sulfide as hydrogen donor. Gaffron found the following relationships:

Utilization of 1 mole of $\text{C}_3\text{H}_7\text{COOH}$: 1 mole of carbon dioxide reduced

Utilization of 1 mole of $\text{C}_6\text{H}_{11}\text{COOH}$: 2 moles of carbon dioxide reduced

Utilization of 1 mole of $\text{C}_7\text{H}_{15}\text{COOH}$: 3 moles of carbon dioxide reduced

Gaffron also found that hydrogen can replace hydrogen sulfide. In the light of Gaffron's findings, van Niel (230) emphasizes the importance of Winogradsky's old experiments with sulfur bacteria, where in the dark hydrogen sulfide is oxidized to sulfur (chemosynthesis). van Niel considers both oxidation of hydrogen sulfide and reduction of carbon dioxide as typical dark reactions also in the case of the purple bacteria and assumes that both in the purple bacteria and in the green plant the oxidation of water represents the process which requires light energy. As a research hypothesis van Niel draws the following picture of the two sorts of photosynthesis:

Both in the plant and in bacterial photosynthesis water is dehydrogenated, giving rise to a reduction of carbon dioxide and a formation of a kind of oxide or oxidation product, perhaps a peroxide. In the plant photosynthesis, the oxidation product formed is spontaneously split into a stable oxide and free oxygen; in the bacterial photosynthesis, the oxide formed is stable and has to be reduced by hydrogen donors like hydrogen sulfide or fatty acids, and consequently no oxygen is formed in this case.

Both the spontaneous liberation of oxygen and the reduction by hydrogen donors restore the group as acceptor of a new molecule of water. van Niel emphasizes that, although for the time being the chances in obtaining the photochemical reaction in cell-free extracts are very small, the chances for an observation of some of the dark reactions in cell-free extracts are much better.

B. Chemosynthesis

Chemosynthesis includes a number of reactions where carbon dioxide is reduced by inorganic or organic compounds; these reactions are independent of light.

The interesting reactions were discovered as long ago as 1890 in the brilliant microbiological investigations by Winogradsky (303, 304). He succeeded in isolating some soil bacteria capable of oxidizing ammonia to nitrite (*Nitrosomonas*) and nitrite to nitrate (nitrobacteria (19)). Winogradsky observed that, in proportion to the oxidation of ammonia, carbon dioxide was reduced to organic compounds according to the following relation: 35 moles of ammonia are oxidized to nitrous acid for 1 mole of carbon dioxide reduced. Meyerhof in 1916 (195) confirmed Winogradsky's equation and found that 105 moles of nitrous acid were oxidized to nitric acid for 1 mole of carbon dioxide reduced. Thus three times as many moles of CO₂ are oxidized in the one-step oxidation of nitrous acid to nitric acid as in the three-step oxidation of ammonia to nitrous acid.

Winogradsky also demonstrated the oxidation of hydrogen sulfide to sulfur in the so-called sulfur bacteria. Carbon dioxide also functions as the hydrogen acceptor in this oxidation.

Woods (309) recently discovered the reversible reduction of carbon dioxide to formic acid by hydrogen gas, and Barker (10) observed the oxidation of ethyl alcohol to acetic acid with the proportional reduction of carbon dioxide to methane. That the methane formed is derived from carbon dioxide has recently (12) been proved directly by means of radioactive carbon dioxide (C¹⁴O₂), which yields radioactive methane (C¹⁴H₄).

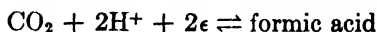
Reactions like the oxidation of ammonia or of hydrogen sulfide by carbon dioxide may look very reasonable from a purely chemical point of view but not from a thermodynamic point of view. The normal potentials of redox systems like ammonia-hydroxylamine or hydrogen sulfide-sulfur are considerably higher than that of the redox system carbon dioxide-formic acid. A very clear illustration of this thermodynamic paradox is the oxidation of alcohol to acetic acid with a stoichiometrical reduction of carbon dioxide to methane. Barker (10) observed the following relationship:



However, E'_0 (pH 7) of the hydrogen-donor system



is -160 millivolts. E'_0 (pH 7) of the hydrogen-acceptor system



is -430 millivolts. How can the negative system serve as hydrogen acceptor for a much more positive system? The explanation must be that the first reduction step of carbon dioxide is carried out by small amounts of strong reducing agents in the cells (for instance, carbonyl groups). The later steps of carbon dioxide reduction,—for instance, of the the reduction of formaldehyde to methyl alcohol or of this substance to methane,—can very well be carried out by alcohol. As soon as acetaldehyde is formed, a reduction of carbon dioxide to formic acid by this substance takes place. The later reductions can be performed directly by alcohol. The interpretation of oxidoreductions between ammonia or hydrogen sulphide and carbon dioxide may be explained in the same manner; the final reduction product of carbon dioxide in these redox processes is, however, not known.

Other kinds of chemosynthesis,—for instance, the reversible reduction of carbon dioxide with hydrogen gas, yielding formic acid, or the uptake of carbonates in the propionic acid fermentation, yielding succinic acid,—have been described in previous sections in this review.

C. The formation of sugar from lactic acid and related compounds

The resynthesis of sugar from lactic acid formed by working muscles (*D*-lactic acid) was first demonstrated by Embden and coworkers (66) in perfusion experiments with mammalian livers. Not only monohexoses but also polyhexoses (glycogen) were formed from lactic acid.

Meyerhof (197) demonstrated a formation of glycogen from lactic acid in muscle tissue, but the liver seems to be the most important organ in carrying out the synthesis of sugar from lactic acid (66, 189). The synthesis of sugar from lactic acid always requires oxygen consumption.

Two main hypotheses have been advanced in order to explain the pathway of sugar formation from lactic acid. Meyerhof supposed that one part (about 20 to 25 per cent) of the lactic acid was completely oxidized by a respiration process which furnishes energy to the resynthesis of sugar from the other part.

Kluyver in 1931 (135) advanced a different hypothesis. According to him, all the lactic acid formed is oxidized in two steps: the first step yields pyruvic acid, which is decarboxylated to carbon dioxide and acetaldehyde; the second step consists in the oxidation of acetaldehyde to glyceraldehyde.

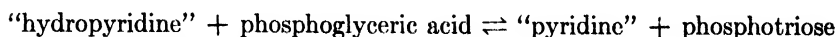
The latter oxidation has been demonstrated in model experiments by Conant and Tongberg (42), who used ceric solution as an oxidizing agent; the glycolaldehyde formed was assumed to be polymerized to sugar. Alkaline solutions of glycolaldehyde actually show slow polymerization of glycolaldehyde into sugar. At the time when Kluyver first discussed this problem, nothing was known about energetic couplings between oxidation-reduction and phosphorylation. Kluyver's theory actually gives a simple and rational explanation of a resynthesis: one part of the molecule is sacrificed as carbon dioxide and the other part is oxidized and polymerized. This coupling is very closely related to the formation of fatty acid from sugar, a process which Kluyver was one of the first to recognize as a mixed dissimilation and assimilation.

Although Kluyver's hypothesis of sugar formation seemed to be the more attractive of the two hypotheses, it has never been supported by enzymatic experiments. Furthermore, the decarboxylation of pyruvic acid into acetaldehyde and carbon dioxide does not occur either in animal tissue or in a number of microorganisms. If carboxylase occurred in animal tissue, ethyl alcohol and not lactic acid would be formed as a result of anaerobic sugar oxidation.

Recent experiments of Hastings, Kistiakowsky, *et al.* (111) with lactic acid containing radioactive carbon in the carboxyl group show that the glycogen formed from such labelled lactic acid contains radioactive carbon only to a very small extent. A much greater amount of the radioactive carbon was found in the carbon dioxide. These recent findings may be a support of the Conant-Kluyver theory. Nevertheless, the lack of carboxylase in animal tissue remains a strong objection against the theory. The rather high radioactivity in the carbon dioxide might also be explained by the new type of decarboxylation-carboxylation recently discovered by Carson and Ruben (34) (see section III, propionic acid fermentation).

Modern enzyme chemistry has rendered much support to Meyerhof's theory. In 1937 Meyerhof and collaborators (207) and at the same time Green, Needham, and Dewan (99) in Cambridge succeeded in the demonstration of what might be called a reverse fermentation. Lactic acid and phosphoglyceric acid in the presence of the pyridine nucleoprotein but without oxygen gave pyruvic acid and phosphotriose; the last was trapped with cyanide or semicarbazide. Meyerhof and coworkers were able to demonstrate this reverse fermentation without trapping the triose, provided they added large amounts of adenylypyrophosphate, and they made the interesting discovery that the added adenylypyrophosphate was dephosphorylated stoichiometrically, so that for every mole of phosphoglyceric acid reduced to phosphotriose 1 mole of phosphate was liberated.

Working with reduced "pyridine," Meyerhof succeeded in the demonstration of a dephosphorylation of adenylypyrophosphate coupled with the reaction

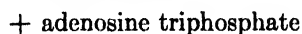
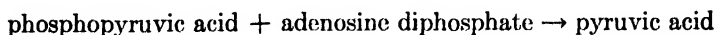


The reverse reaction:



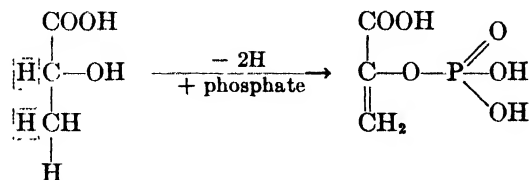
is coupled with a phosphorylation of adenylic acid to adenylypyrophosphate (206). Meyerhof explained these two facts in the following way: the oxidoreduction of glycolysis furnishes energy for the phosphorylation of adenylic acid, whereas the dephosphorylation of adenylypyrophosphate furnishes energy to the reverse oxidoreduction, i.e., for the sugar formation from lactic acid. Under natural conditions adenylypyrophosphate occurs only in small amounts and therefore has to be rephosphorylated by energy-furnishing redox processes; this rephosphorylation of adenylic acid is one of the important tasks of the respiration. Although the clear demonstration of these step reactions, proceeding in the reverse direction of those in the glycolysis, represented the first step towards a chemical understanding of sugar synthesis from lactic acid, two problems remained still unanswered. The first was the formation of phosphoglyceric acid from pyruvic acid; the second the nature of the coupling between dephosphorylation and oxidoreduction. The first problem is partly solved, the other completely.

Formation of sugar from lactic acid is a reverse glycolysis, which means that the hydroxyl group of lactic acid is the hydrogen donor and the carboxyl group of phosphoglyceric acid the hydrogen acceptor; the latter substance is regenerated steadily from the precursor, phosphopyruvic acid, by the addition of water. The missing link in the scheme is the phosphorylation of pyruvic acid to phosphopyruvic acid; this process has never been demonstrated experimentally. Furthermore Meyerhof and collaborators, (204) on the basis of some experiments with radioactive phosphate, assume that the well-known reaction



goes in one direction only. Of importance for this problem, however, is the formation of phosphopyruvic acid from malic or fumaric acid added to kidney extracts (Kalekar (123)). Fumaric acid or malic acid was oxidized in the presence of fluoride and gave rise to an accumulation of phosphopyruvic acid which could only be formed by an oxidation of malic acid, since fluoride prevents a formation of this ester from sugar. Ferd-

man (79) has recently been able to demonstrate a corresponding formation of phosphopyruvic acid from lactic acid in muscle tissue. Presumably phosphopyruvic acid is formed by an oxidation of lactic acid by the following reaction:

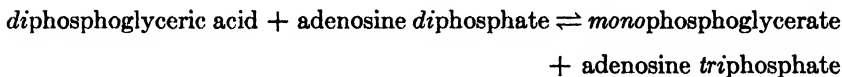


Lactic acid

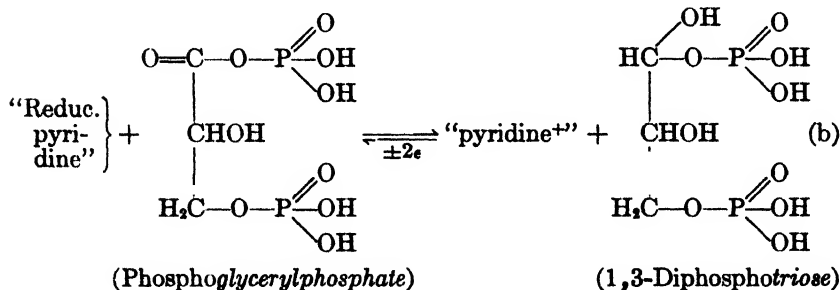
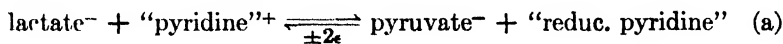
Phospho(enolic)pyruvic acid

Whether the phosphate enters the lactic acid or, simultaneously with the oxidation, enters the oxidation product, phospho(enolic)pyruvic acid, is unknown. A corresponding reaction with a preliminary formation of phosphoöxaloacetic acid is probably the mechanism of the phosphopyruvic acid formation from malic and fumaric acids. Addition of phosphate instead of water to fumaric acid would yield phosphomalic acid (*cf.* Lipmann (175)).

The nature of the coupling between the reduction of phosphoglyceric acid and adenylypyrophosphate dephosphorylation was not understood until Warburg and his pupils (299, 220) separated the different enzymes and obtained the new labile 1,3-diphosphoglyceric acid (*cf.* section V). The diphosphoglyceric acid reacts with the adenine nucleotide in the reversible reaction



The diphosphoglyceric acid accepts the electrons from the reduced "pyridine." The diphosphotriose formed splits to monophosphotriose and phosphate. The reverse glycolysis proceeds therefore as follows:



The phosphorylation of the carboxyl group by adenylypyrophosphate makes a reduction of this group thermodynamically much easier, since the potential of the aldehyde-carboxyl system after a phosphorylation is raised approximately 200 millivolts; the reasons for such a shift in the potential have been given in section V. Thus a phosphorylation of the carboxyl group creates a relatively good hydrogen acceptor. The carboxyl phosphate is reduced to aldehyde-phosphate, which immediately liberates the phosphate. The result of these reactions is that adenylypyrophosphate is dephosphorylated and monophosphoglyceric acid is reduced to monophosphotriose; this over-all reaction is identical with Meverhof's equation.

The reduction of carboxyl groups to carbonyl groups is thermodynamically difficult to carry out in biological systems, because in such systems the carbonyl-carboxyl redox system is the strongest reducing system of all. According to Wieland's theories (302), aldehydes before dehydrogenation take up water to form aldehyde hydrates, which become the hydrogen donor proper; a reverse reaction, i.e., a reduction of carboxyl to aldehyde hydrate, is thermodynamically very unlikely (E'_0 approximately -450 millivolts). According to Warburg (299), the aldehyde group of triose (and according to Lipmann (175) the carbonyl group in pyruvic acid) takes up phosphoric acid (carbonyl phosphate) before biological dehydrogenation; the carbonyl ester thus formed represents the hydrogen donor proper. A reverse reaction, i.e., reduction of the carboxyl phosphate of diphosphoglyceric acid to carbonyl phosphate by the reduced pyridine nucleotide, is thermodynamically a very likely reaction.

Thus replacement of water by phosphate converts processes which would have been highly irreversible into more reversible³⁰ processes and thereby prevents scattering of energy as heat. We meet the same feature again in the cleavage of polysaccharides which can be split by water (diastatic hydrolysis, irreversible) or by phosphate (Cori's phosphorolysis, reversible); these last reactions will be described in the next section.

D. The synthesis of polyhexoses from monohexoses

The liver and the muscles are able to build up polyhexoses, mainly glycogen, from the monohexoses, glucose and fructose, provided oxygen is present. Until very recently the formation of glycogen has been demonstrated only in an intact liver perfused with oxygenated blood or in liver slides shaken in oxygenated saline solution. It was a general belief among physiologists that, although reactions like phosphate esterifications can be demonstrated in cell-free extracts (or even with crystalline proteins), the formation of glycogen is a biological manifestation which is so closely

³⁰ Reversible in a thermodynamic sense.

connected with the living intact cell structure that one would be very unlikely to find glycogen formation in extracts, not to speak of purified enzymic systems. Owing to the brilliant work of Cori and Cori and collaborators, the mechanism of the breakdown and the formation of glycogen has now been recognized.

In order to understand the mechanism of glycogen formation, one has to be acquainted with the investigations on the breakdown of glycogen. For a long time it has been the general belief that the only biological way of breaking down polyhexoses (starch, glycogen, dextrins) was an ordinary hydrolysis catalyzed by certain enzymes, called amylases or diastases. These enzymes which occur in the digestive tract were also considered responsible for the liberation of glucose (blood sugar) from the liver; the liver, however, does not contain diastase in large amounts, and probably all activity is due to diastase in the blood plasma. Since diastase, even in the presence of very high amounts of glucose, cannot catalyze glycoside formation, the formation of glycogen from glucose cannot be due to a reverse diastase action.

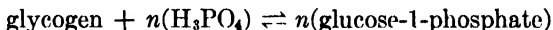
In 1935 Cori and Cori (36) found that glycogen in intact muscles takes up inorganic phosphate, and the same year Parnas and Ostern (238) found the same phenomenon in aged and dialyzed muscle extract; the product formed was found to be a 6-monophosphate. Since the oxidative enzymes are inactivated or removed, the uptake of phosphate is not coupled with an oxidation. The product formed by the phosphorylation is the well-known mixture containing 70 per cent of glucose-6-monophosphate and 30 per cent of fructose-6-monophosphate (Embden ester). In 1936 Cori and Cori (47) made the important discovery that the primary product of the glycogen phosphorylation in muscle extract is glucose-1-phosphate, i.e., a hexose phosphate phosphorylated on the aldehyde group.

The glucose phosphate (Cori ester) exhibits the following properties: no reduction in a non-enzymatic system, since the aldehyde group is phosphorylated; strong dextrorotation, $[\alpha]_D^{20} = +120^\circ$; and high lability to acid hydrolysis (5 min. boiling in 1 *N* hydrochloric acid liberates all the phosphate). Cori, Colowick, and Cori (46) furthermore succeeded in a chemical synthesis of glucose-1-phosphate. Glucose was acetylated and brominated; the bromoacetylglucose was heated with silver orthophosphate. The triacetylglucose monophosphate thus formed was subjected to a mild acid hydrolysis, yielding 1 mole of glucose-1-phosphate per mole of triacetylglucose monophosphate. Both the 1-ester isolated from muscles and the chemically prepared 1-ester are rapidly converted to glucose-6-phosphate by a specific enzyme occurring in all tissue (Cori (44)). The enzyme requires small amounts of magnesium ions. The conversion

of the 1-ester to the 6-ester is irreversible, i.e., the addition of the 6-ester to this enzyme does not yield the 1-ester.³¹

The discovery of glucose-1-phosphate represents the introduction to a new chapter in the study of carbohydrate metabolism.

Cori, Cori, and Schmidt (48, 52) and Kieessling (130) in 1939 succeeded in the separation of the enzyme which converts glycogen into the 1-ester from the enzyme which converts the 1-ester into the 6-ester. The purification of this enzyme bore great fruit, because it enabled both Kieessling and Cori and collaborators to demonstrate a new and very interesting enzymatic equilibrium reaction:

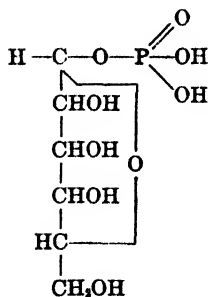


where the equilibrium is very much to the side of glycogen; about 80 per cent of the 1-ester is converted into glycogen. The chemically prepared 1-ester is just as active as the enzymatically prepared 1-ester. Adenylic acid acts as coenzyme (*cf.* section IV). The equilibrium constant is dependent on the concentrations of inorganic phosphate and 1-ester but is independent of the glycogen concentration, presumably because glycogen is a substance of high molecular weight or is in colloidal dispersion acting essentially as a solid saturating body.

Cori (45), as well as Parnas, point out that the rôle of phosphate in the cleavage of glycogen to glucose-1-phosphate corresponds to that of water in the hydrolysis of glycogen into glucose. In the first case the glycoside

linkages are split by $\text{HO}-\text{P}(\text{OH})_2$; in the other by $\text{HO}-\text{H}$. The cleavage

31



Glucose-1-phosphate (Cori ester)

It is worth while to notice that the phosphate in the glucose-1-phosphate is linked to a carbon atom in the "Haworth ring." The hydroxyl group of the 1-carbon atom is an acid group which forms salts, for instance, calcium glucosate.

of glycogen by phosphate is therefore called phosphorolysis in analogy to hydrolysis, the splitting by water. The replacement of water by phosphate makes the reaction reversible (*cf.* the oxidation of glyceraldehyde).

Experiments made by Cori and Cori (50) with purified enzyme preparations from brain and muscle tissues showed two interesting new phenomena: (1) The enzyme purified from muscle tissue gives rise to the formation of a polyhexose, which, like starch, gives a blue color reaction with iodine and shows the same Debye diagram as potato starch. (2) In the polymerization of 1-phosphoglucose to glycogen, the addition of glycogen is necessary to start the reaction.

Cori and Cori (51) recently analyzed this glycogen activation in detail; they found 1.4 mg. of glycogen per 100 cc. of reaction mixture sufficient to give a prompt abolishment of the lag period. Muscle phosphorylase activated by 10 mg. per cent glycogen exhibits a marked decrease in activity after 20 min., in spite of the fact that a large amount of newly formed starch (140 mg. per cent) is present. The newly formed starch therefore has much less "autocatalytic" effect than the glycogen added. This phenomenon, together with the fact that the starch formed by muscle phosphorylase is firmly bound to the proteins, indicates that the starch blocks the surface of the enzyme and limits the amount of polysaccharide which can be synthesized per enzyme molecule. The polysaccharide formed by liver and brain is closely related to ordinary glycogen, i.e., gives a brown color with iodine, is not firmly bound to the proteins, and has an autocatalytic effect on polysaccharide formation. The rate of polysaccharide formation is raised considerably by increasing the amounts of glycogen added. Cori and Cori find that the effect of added glycogen corresponds to that of a coenzyme. In an experiment which contained 15 mg. of protein per 100 cc., the concentration of glycogen which gives one half of maximal velocity was 27.6 mg. per 100 cc. The authors point out that one molecule of glycogen (assuming for it a molecular weight >250,000) could hardly be bound to one enzyme molecule. Probably many enzyme molecules form one unit of glycogen or starch from units of twelve to eighteen glucose molecules.

The thermodynamics of the conversion of 1-phosphoglucose to 6-phosphoglucose is of considerable interest, since this reaction seems to be practically irreversible. This means that a phosphorylation of the aldehyde group requires at least a couple of thousand calories more than a phosphorylation of the 6-hydroxyl group. A direct phosphorylation of the aldehyde group with inorganic phosphate, which apparently takes place in the oxidation of glyceraldehyde by pyridine enzymes, seems hardly possible in glucose, presumably owing to the "Haworth ring."²²

²² *Cf.* also the missing reaction between hexoses and sulfite.

Perhaps the pyrophosphate energy is of importance also for the formation of 1-phosphoglucose.

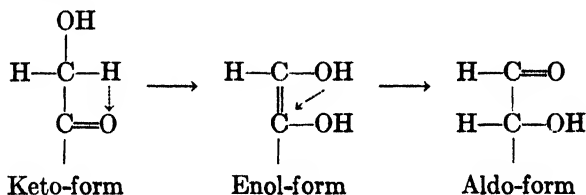
Thus the reaction which requires most energy is the phosphorylation of glucose to 1-phosphoglucose; the 1-ester thus formed is polymerized without cost of energy. It is important to bear this fact in mind, because the formation of polymerized products in living systems until very recently has been considered as synthesis "par excellence."

The Cori-Kiessling equilibrium is, as pointed out in the introduction, a clear illustration of a thermodynamically spontaneous polymerization. The "expensive" part is the formation of the precursor substance, in this case 1-phosphoglucose, which then polymerizes without further cost or even with a small liberation of energy.

A phosphorylation of glucose to glucose-1-phosphate has so far only been demonstrated indirectly in liver slices. Ostern and coworkers (236) have found in recent experiments with liver slices that calcium is necessary for the synthesis of glycogen from glucose. These investigators are inclined to think that the importance of calcium for glycogen formation is due to a depression of the phosphate concentration.

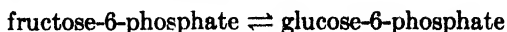
E. The conversion of fructose to glucose

The conversion of fructose to glucose takes place with great rapidity in the liver. The mechanism of this reaction is not entirely known. In alkaline solution a slight and very slow conversion from fructose to glucose takes place. It is generally assumed that the conversion of the ketohexose to the aldohexose has to pass an enolic step:



The conversion of fructose to glucose in the liver is a rather complicated process which requires tissue respiration. If, however, the fructose is phosphorylated in the 6-position, the enzymatic conversion into glucose-6-phosphate is a simple reversible process which is catalyzed by a special enzyme (Lohmann (180)).

The equilibrium of the reaction:



is approximately two-thirds glucose ester and one-third fructose ester. Liver tissue dephosphorylates the glucose phosphate much faster than the

fructose phosphate (53, 92). The phosphorylation of fructose is the only step which requires respiration.

F. The action of dinitrophenol

Recent work in microbiology has brought to light many cases in which a metabolite added to bacteria has been synthesized to cell constituents simultaneously with an oxidation of another part of the added metabolite.

If, for instance, a certain amount of glucose is added to aerobic microorganisms, about one-third to one-half is converted to a cell constituent (either sugar, protein, or fatty acid). Clifton and Logan (38) made the interesting observation that the addition of dinitrophenol, even in such low concentrations as $m/16,000$, converts the metabolism of the bacteria to a pure combustion metabolism, i.e., the oxygen consumption corresponds to the complete oxidation of all the glucose added. Recently Douderoff (61), in van Niel's laboratory, made the important observation that pyruvic acid is accumulated in the presence of dinitrophenol; pyruvate was isolated as the dinitrophenylhydrazine compound. The accumulation of pyruvic acid in bacteria poisoned with dinitrophenol is surprising. The phenomenon indicates that phosphoglyceric acid formed in the normal metabolism is reduced to triose phosphate by pyruvic acid. An inhibition of this reduction would give a temporary accumulation of the equilibrium ester: phosphoglyceric acid \rightleftharpoons phosphopyruvic acid.

IX. THE SIGNIFICANCE OF PHOSPHORYLATION IN LIVING CELLS

A. The occurrence of phosphorylation in living cells

Most studies on phosphorylations have been carried out in cell juices or extracts, where the lack of enzymes or the inhibition of enzymes leads to the accumulation of phosphoric esters in such amounts that they can be isolated. In tissue slices or intact organs the accumulation of phosphoric esters usually is smaller. In intact muscles very large amounts of creatine phosphate and adenylypyrophosphate occur, and in the introduction it has been described how this storage of phosphoric esters decreases rapidly during contractions. When working muscles are poisoned with iodoacetic acid ($m/10,000$) a marked increase in the amount of hexose phosphates takes place (Lundsgaard (185)). Cori and Cori (49) found that epinephrine, even in extremely small amounts, when added to muscles gives a marked increase in the amount of hexose monophosphate.

Distinct accumulations of hexose phosphates have also been demonstrated in the living yeast cell during the fermentation of sugar (McFarlane (193)), in microorganisms (306), and in the perfused intestine during the absorption of glucose or fructose (Lundsgaard (188)).

Even in cases where a formation of phosphoric esters cannot be observed, such a formation might very well take place. It must be borne in mind

that phosphoric esters are intermediate products in metabolism, just like ketone bodies or pyruvic acid, and can therefore not be expected to accumulate under normal conditions. This is also illustrated by the experiments with kidney extract with and without fluoride: in the presence of fluoride a large accumulation of hexose diphosphate occurs; in the absence of fluoride the accumulation is very small.

One of the objections against the assumption that hexose phosphates are oxidized also in intact cells is based on the observation that tissue slices (for instance, brain tissue) utilize glucose but neither hexose monophosphate nor hexose diphosphate. This phenomenon is, however, explained simply by the fact that only free sugars are able to pass the cell membrane. Thus the free sugar is the transport form,³³ and the phosphorylated sugar is the form in which it is oxidized. That sugar also can be oxidized in the unphosphorylated form appears from the experiments of Müller and of Franke and Deffner (87) with the glucose dehydrogenase from molds and those of Harrison with the glucose dehydrogenase from liver.

A number of investigators who held the view that phosphate does not play any rôle in the metabolism of intact cells referred to some important observations made by Nilsson and Alm in 1936.

Nilsson and Alm (231) describe the preparation and properties of a new sort of dried yeast which, since it is dried much faster (less autolysis) than the ordinary dried yeast (Lebedew yeast), contains more intact enzyme systems than the Lebedew yeast and therefore shows a metabolism much more like living yeast cells, although the membranes of this dried yeast are more or less digested. If glucose is added to ordinary slowly dried yeast (Lebedew yeast), the fermentation does not start immediately but only after a so-called induction period, presumably because of the slow formation of hexose diphosphate before any energy-spending oxidoreduction has started. Addition of minute amounts of hexose diphosphate and acetaldehyde starts the fermentation immediately (*cf.* Meyerhof (199)). Furthermore, Lebedew yeast accumulates hexose diphosphate according to Harden and Young's equation and requires the extra addition of orthophosphate as soon as all the inorganic phosphate has been esterified. Nilsson yeast, on the other hand, has only a very brief induction period, ferments glucose more rapidly, and exhibits no accumulation of phosphoric ester, thus being independent of the extra addition of inorganic phosphate, just like living intact yeast cells. Nilsson and Alm found, in addition, that inorganic phosphate, added to a suspension of quickly dried yeast (Nilsson yeast), gives no stimulation of the fermentation but even an inhibition which, however, does not appear before half of the glucose has been fermented. Furthermore, the addition of phosphate to Nilsson

³³ Peters *et al.* have shown that vitamin B₁ penetrates the cell membrane much more quickly than does vitamin B₁ pyrophosphate.

yeast gives an accumulation of hexose diphosphate in the first half of the fermentation period. When half of the glucose has been fermented, the accumulation of hexose diphosphate culminates. The rather complicated observations are illustrated in curves taken from one of Nilsson's publications (figure 1, from reference 231, page 259). The factor which is present in Nilsson yeast and which is necessary for the occurrence of an alcoholic fermentation of the same type as that of living yeast (i.e., according to the Gay-Lussac equation: $\text{glucose} = 2 \text{ ethyl alcohol} + 2 \text{ CO}_2$) is very thermolabile and is inhibited by inorganic phosphate.

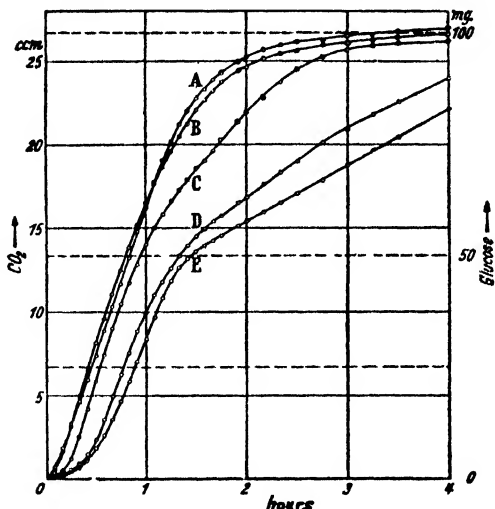


FIG. 1. Fermentation of glucose by Nilsson yeast. Total volume = 2 cc.; 200 mg. of dry yeast; 100 mg. of glucose 0.67 molar phosphate (pH 6.4) added. 0.84 cc. of phosphate solution equivalent to 100 mg. of glucose. Temperature, 30°C. Curve A, without extra phosphate added; curve B, 0.25 cc. of 0.67 molar phosphate solution added; curve C, 0.50 cc. of 0.67 molar phosphate solution added; curve D, 0.75 cc. of 0.67 molar phosphate solution added; curve E, 1.00 cc. of 0.67 molar phosphate solution added.

Recently Lipmann observed a very similar phenomenon in maceration juice from bakers yeast; here the fermentation proceeds according to the Harden-Young equation until approximately 80 per cent of the phosphate is esterified; the fermentation then proceeds according to the Gay-Lussac equation without further decrease of the inorganic phosphate, until all the sugar is fermented.

B. The apparent absence of phosphorylation in living yeast

The phosphate effect discovered by Nilsson and Alm has, as mentioned

before, led a number of biologists to believe that phosphorylations do not occur in living cells. Nilsson himself did not interpret his observations in this direction, however. He assumed that sugar was monophosphorylated and then split into 1 mole of triose and 1 mole of triose phosphate; the unphosphorylated triose was assumed to be the substance which is fermented. Nilsson and Alm's observations can, however, also be interpreted in a quite different way, which has the great advantage of making it possible to correlate their findings with the modern concepts of sugar oxidation; in particular, with Negelein and Brömel's discovery of the diphosphoglyceric acid described in section V. Nilsson and Alm point out that the difference between the fermentations in quickly and in slowly dried yeast must be attributed to the presence of a phosphatase in the first kind of yeast and the absence of this phosphatase in the latter kind. The extraordinary thermolability of this phosphatase, in connection with its sensitivity to high phosphate concentrations, indicates the identity of the phosphatase with the so-called adenylypyrophosphatase, i.e., with the specific enzyme which catalyzes the liberation of orthophosphate from the organic phosphate ester. The enzyme was first observed in extracts of liver tissue (Jacobsen (117)), but occurs also in kidney and muscle tissue. This enzyme is not only inhibited by inorganic phosphate (13) but shows also an extraordinary sensitivity to even very moderate rises in the temperature (69). The assumption that adenylypyrophosphatase occurs in Nilsson yeast but is absent from Lebedew yeast would therefore be able to account for most of the facts.

Actually, Lebedew yeast and Lebedew juice do not contain adenylypyrophosphatase; it would be of considerable interest to investigate whether rapidly dried yeast contains adenylypyrophosphatase and if addition of this enzyme will convert the "juice fermentation" (characterized by the conversion of 50 per cent of the sugar to carbon dioxide and alcohol and the other 50 per cent to hexose diphosphate) into the fermentation type of the living yeast which converts 100 per cent of the sugar into carbon dioxide and alcohol.³⁴

In any case, however, it must be obvious that the large accumulation of phosphoric esters observed in different extract systems is an artifact due to inhibition or lack of some enzyme systems.

C. The phosphate cycle

The classical cycles of Parnas and Meyerhof account for the phenomena observed in yeast juice of muscle extracts poisoned with fluoride. For every mole of triose phosphate converted into alcohol or lactic acid, 2

³⁴ In order to carry out such an experiment, the adenylypyrophosphatase preparations which are very impure have to be subjected to fractionation.

moles of phosphate are used to phosphorylate sugar, which means that 2 moles of triose phosphate arise per mole of triose phosphate oxidized. This gives an accumulation of 1 mole of triose phosphate per mole of lactic acid formed or 1 mole of hexose diphosphate per mole of glucose converted into alcohol and carbon dioxide, which actually is the Harden-Young equation.

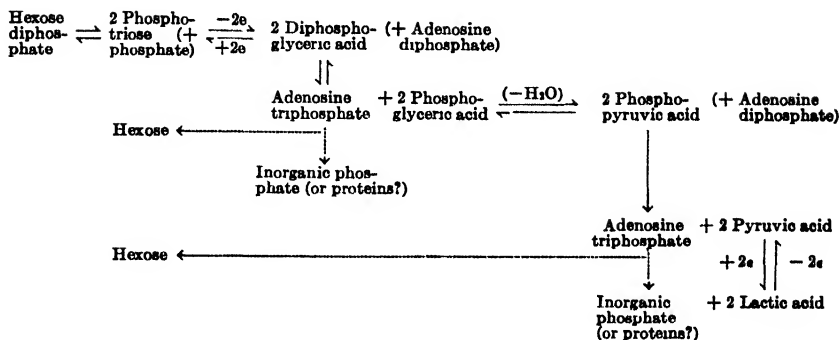
The lack of adenylypyrophosphatase in yeast juice means that adenylypyrophosphate can be dephosphorylated only by a phosphate acceptor, such as glucose.

The necessity of phosphate acceptors for the alcoholic fermentation and for the glycolysis in cell-free systems has been very clearly demonstrated in the brilliant experiments of Meyerhof.³⁶ These experiments are significant for the interpretation of a number of observations where cell-free systems ferment unphosphorylated sugars better than phosphorylated sugars. Meyerhof (199) showed that the reason why hexose diphosphate is not fermented by Warburg and Christian's purified fermentation system is that no phosphate acceptor is present. Geiger (90) found that brain extracts ferment glucose and fructose more than ten times faster than the corresponding mono- or di-phosphates which, however, are accumulated during the glycolysis of glucose and fructose. Recent experiments by Ochoa show, however, that brain extracts form large amounts of lactic acid, both from hexosemonophosphate and from hexosediphosphate. Warburg and Christian (296) observed an interesting effect of glucose and fructose on oxidation; the oxidation of phosphohexonic acid proceeds to completion only in the presence of fructose or glucose. The nature of the action of fructose is unknown.

It is very likely that in the living cell the same reactions occur as in extracts, with the difference that in living cells only 1 mole of phosphate is used for esterification of sugar per mole of triose phosphate oxidized; the other mole of phosphate is liberated as inorganic phosphate, presumably by the activity of adenylypyrophosphatase. This seems also to be the case in extracts of brain (Ochoa: *J. Biol. Chem.*, in press). (See diagram at top of page 161.)

If all the phosphate goes back to hexose, 2 moles of hexose diphosphate are formed for every mole of hexose diphosphate fermented; this will give an accumulation of 1 mole of hexose diphosphate per mole of hexose diphosphate fermented, i.e., the type of fermentation taking place in Lebedew juice and expressed in Harden and Young's equation.

³⁶ Belitzer (17) has recently demonstrated a marked stimulation of the respiration of muscle pulp by creatine, during which creatine is phosphorylated to creatine phosphate. This is the first demonstration of what might indicate a compulsory coupling between respiration and phosphorylation in animal tissue.



D. The "break" in the phosphate cycle

Needham and Phillai (218) and Meyerhof and coworkers (205) have shown that half of the phosphate taken up in the oxidation can be transferred to creatine. The phosphate in the intact working muscle, however, is not only transferred to sugar and creatine but also liberated as inorganic phosphate, since muscular contraction leads to a great increase in the inorganic phosphate and decrease in phosphocreatine.

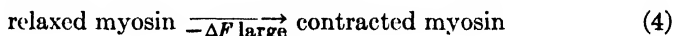
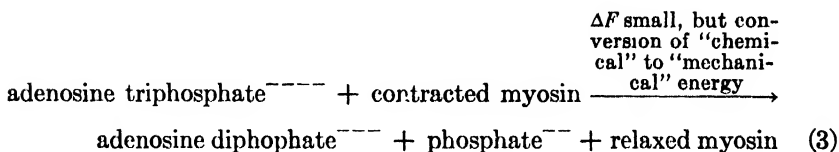
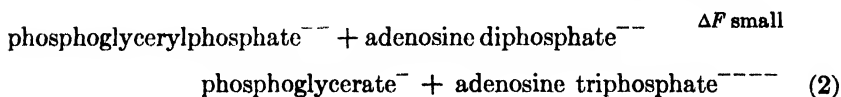
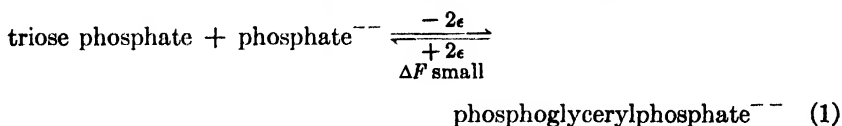
As pointed out by D. M. Needham (217) (*cf.* also Lundsgaard (187)), this break in the phosphate cycle very likely represents the transmission of the phosphorylation energy to the contractile system. As mentioned before, the dephosphorylation of adenylypyrophosphate represents a liberation of free energy which is larger than most of the biological step reactions ($\Delta H = 11,000$ calories per phosphorus atom). It is most unlikely that such a large amount of energy should merely be scattered as heat, as would be the case if a plain liberation of inorganic orthophosphate took place.^{35a} The only way in which pyrophosphate energy can be used is by a primary reaction of the adenylypyrophosphate with the contractile protein, myosin.³⁶ Since exhaustion of the adenylypyrophosphate storage in the iodoacetate-poisoned muscle is accompanied by rigor (Lundsgaard (185)), adenylypyrophosphate dephosphorylation may be coupled to the relaxation (recharging) of the myosin system. Thus, there is reason to believe that in the living cell adenylypyrophosphate is not dephosphorylated directly but through cellular structures, acting as phosphate-transfer systems.

^{15a} Cf. the discussion of the sugar phosphorylation by pyrophosphate (section V, D).

²⁶ Of interest in this connection is the reaction between metaphosphate and albumin (Perlmann and Herrmann (243)). Albumin and metaphosphate form, at the acid side of the isoelectric point of the protein, a crystalline compound. The crystalline metaphosphate-protein is soluble in dilute salt solutions. The metaphosphate was found linked to the basic groups of the protein.

If we suppose that myosin in the contracted state acts as a phosphate acceptor and during the relaxation process as a phosphate donor, then we would get an illustration of how changes in cellular structures are able to "regulate" metabolic processes.

The coupled reactions might be illustrated as follows:



The more contracted the myosin, the greater the "consumption" of adenosine polyphosphates and the oxidation³⁷ of triose phosphate. Thus, according to these considerations, a contraction of myosin starts the oxidation of phosphotriose to pyruvate and of the latter to the acetate level.

The sudden increase in respiration (or, in the absence of oxygen, in lactic acid formation) succeeding a muscle contraction is an old observation. Recent studies of Millikan (216), who measured the rate of reduction of myoglobin in rest and during contraction, show that the increase in oxygen consumption appears less than $\frac{1}{2}$ second after the contraction starts.

The dephosphorylation of creatine phosphate, the other reaction which rephosphorylates adenylic acid, appears much later than the oxygen consumption. It must, however, be borne in mind that a sensitive method for measuring creatine phosphate hydrolysis, corresponding to Millikan's method of estimation of the rate of the oxygen consumption, does not exist.

The modern concept of biological oxidations and phosphorylations will probably be able to give a real chemical explanation of the fundamental biological phenomenon that changes in cellular structures as, for instance, the contraction and relaxation of myosin, are able to regulate oxidations.

If carbonyl groups were oxidized according to the old Wieland scheme

³⁷ Cf. the experiments of Belitzer (17), showing that the respiration of muscle pulp is stimulated considerably by addition of the phosphorus acceptor, creatine.

(i.e., by uptake of water, forming carbonyl hydrates which then were oxidized to the stable carboxylate structure), the free energy would be liberated during the oxidation (*cf.* table 1) and therefore scattered as heat. Such a reaction has a very high degree of irreversibility (167) and the rate of such a kind of oxidation must therefore remain unaffected by changes

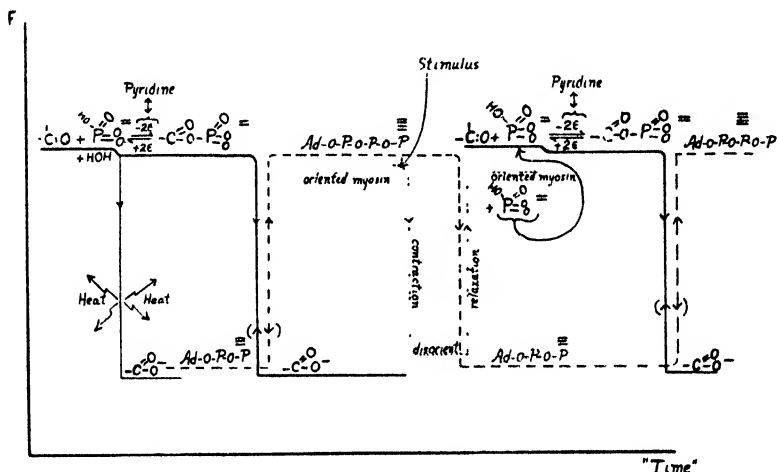


Fig. 2. This figure shows how metabolism and changes in myosin might be coupled. The balance shows only that when myosin in the muscle contracts and relaxes, carbonyl groups are oxidized. The single steps in this cellular coupling can be detected only by purification and separation of enzymes. C=O , the carbonyl

group of sugars or pyruvic acid; $\text{—}\overset{\text{O}^-}{\text{C}}=\text{O}$, the carboxylate ion of sugar acids, or lower

fatty acids; $\text{—C—O—P}\begin{matrix} \text{O}^- \\ \parallel \\ \text{OH} \end{matrix}$, the carboxyl phosphate of glyceryl phosphate or

acetyl phosphate; Ad-O-P-O-P--- represents adenosine diphosphate and Ad-O-P-O-P-O-P--- represents adenosine triphosphate (the number of negative charges is in accordance with Lohmann (182)); F , free energy (the relative changes in free energy are arbitrary). —, energy of metabolite; ---, energy of adenine polyphosphate. The electrons ($\pm 2e$) are accepted by the pyridine nucleotide or furnished by the reduced pyridine nucleotide.

in cellular structure. One of the important trends at present in enzyme chemistry is toward the study of the complex dephosphorylations which occur during and after muscular contraction.

It is hardly necessary to emphasize how difficult and complicated an experimental demonstration of the restitution of myosin by adenylypyrophosphate (reaction 3) will be. Even if a phosphorylation of myosin *in*

vitro should be successfully demonstrated, we are far from an understanding of the relaxation process. This process, which is supposed to be accompanied by a liberation of phosphate and which represents the conversion of chemical into mechanical energy, must be connected with a secondary structure which stabilizes the "recharged" myosin.^{37a} The stimulus is supposed to proceed in this secondary structure and to abolish the stabilizing effect, thus causing an immediate "discharge" in the myosin.

E. The mechanism of sugar absorption

It has been postulated that absorption of sugars in intestine and in kidney cortex requires a transitory phosphorylation of sugar (186, 282). A biological transformation of sugar into a sugar phosphoric acid ester would keep the sugar concentration in the cell at an extremely low level and would therefore permit a steady diffusion of sugar from the intestinal tube or kidney tubules into the cell. The sugar phosphoric acid ester was supposed to be rapidly dephosphorylated at the other end of the cell, making a rapid diffusion of the liberated glucose and phosphate into the blood stream possible. This scheme actually illustrates how sugar can be transferred from the intestine or kidney tubules to the blood, even in cases where the sugar concentration in the intestine or kidney tubules is lower than in the blood (120). It has for a long time been known that membranes which are able to transfer salt or organic compounds against a gradient of concentration require oxygen, apparently because the absorption is coupled with energy derived from cell respiration.

Is there any experimental support for the theory that the driving force in the absorption of sugars is connected with a phosphorylation-dephosphorylation cycle? The enzymes required for such reactions as phosphorylation-dephosphorylation actually occur in large amounts in intestinal mucosa and kidney cortex. In the animal organism three tissues contain large amounts of phosphatases,—the ossification centers in cartilage, the intestinal mucosa, and the kidney cortex. Robison (249) discovered the occurrence of large amounts of phosphatases in cartilage in the phase when the calcification appears. He very clearly demonstrated that the function of the phosphatases in this tissue is a precipitation of calcium phosphate from the soluble calcium hexosemonophosphate. In his monograph (249) Robison discusses the significance of the phosphatases in kidney and intestinal mucosa: . . . "the kidney phosphatase may be concerned in the normal secretion of phosphates *in vivo* . . .".

On the basis of two well-known facts, Lundsgaard (186) in 1933 advanced

^{37a} The so-called "relaxed" or "recharged" myosin corresponds in many respects to stretched rubber; the heat capacity is decreased as a consequence of the increased orientation (cf. H. Mark and K. H. Meyer).

his hypothesis that phosphorylation was involved in the mechanism of sugar absorption. He started with the question whether there is any connection between (1) the occurrence of high phosphatase activity in both kidney cortex and intestinal mucosa and (2) the fact that these same two tissues absorb glucose.

A glycoside, phlorhizin, inhibits the absorption of glucose both in kidney and in intestinal mucosa and the same glycoside strongly inhibits the phosphorylation of polyhexoses in muscle extracts (186). Later the rapid phosphorylation of glucose and fructose in kidney cortex was discovered (119) and the action of phlorhizin on this system was demonstrated. The phosphorylation system in kidney cortex is actually very sensitive to phlorhizin ($M/500$ gives 80 to 90 per cent inhibition (120)). Recently Lundsgaard (188) in perfusion experiments demonstrated a marked accumulation of phosphoric esters in the intestinal mucosa when glucose and fructose are absorbed. The accumulation of phosphoric esters was greatest when fructose was absorbed. The classical work of Cori (43) showed that fructose is absorbed more slowly from the intestinal tract than glucose and galactose. Since fructose is rapidly phosphorylated, the slow absorption of fructose may be due to a delayed dephosphorylation (121). This assumption would at least explain the great accumulation of hexose diphosphate during an absorption of fructose. It is known that both fructose monophosphate and fructose diphosphate are dephosphorylated much more slowly than glucose monophosphate. Lundsgaard emphasizes, however, that so far we are unable to decide whether the accumulation of phosphate esters during sugar absorption is due to the absorption or merely represents an increased metabolism.

Since galactose is so rapidly absorbed from the intestine (43), it would be of great importance to demonstrate that a rapid enzymatic phosphorylation of galactose takes place in intestinal mucosa.

Lundsgaard's hypothesis is supported by microchemical studies of the kidney cortex, which show that glucose absorption (288), phlorhizin accumulation (63), and phosphatase activity (93) are confined to the same structure in the kidney cortex, the proximal tubules. Kidneys without a filtration system, i.e., aglomerular kidneys (for instance, from the toad fish), contain much less phosphatase than glomerular kidneys from closely related species (Kalckar, 1940; unpublished work).

X. COÖRDINATION BETWEEN OBSERVATIONS MADE *in vitro* AND *in vivo*

Is it possible to reconcile the results obtained from enzyme studies with those obtained from experiments on living cells?

The application of results obtained from *in vitro* experiments to the explanation of *in vivo* phenomena requires very careful consideration.

We have, however, numerous illustrations of how successful such attempts can be. As pointed out by Green (97), Keilin's studies of the cytochromes offer a classical example of a perfect reconciliation of *in vitro* and *in vivo* studies. The first studies of cytochrome by MacMunn and by Keilin were *in vivo* experiments, in which the absorption lines were observed in the wings of insects and in yeast cells. Later studies by Keilin, Theorell, and Ogston and Green showed that the properties of the isolated cytochrome *c* are identical with those of the cytochrome in the tissue. The demonstrations of flavin and nicotinic acid as the essential components of oxidation enzymes (Warburg and collaborators) added greatly to the significance of the identification of these two compounds as essential growth factors (vitamins).

The enzymatic experiments of D. M. Needham, Meyerhof, and others make it possible to account for almost every chemical process occurring in contracting muscles. The results of Lundsgaard's investigations of normal and iodoacetate-poisoned muscles are very well interpreted by the recent enzymatic work of Needham and Meyerhof.

The use of isotopes in *in vivo* experiments, introduced in 1924 by Hevesy, is of great value for a coördination of "vivo" and "vitro" results. This method is able to illuminate reactions which cannot be detected *in vivo* in any other way.

It has been stated earlier in this review that the use of radioactive carbon in enzymatic experiments may reveal the pathway of some important biological syntheses (photosynthesis; carbohydrate synthesis from lactic acid). The use of radioactive phosphorus may also be of value in harmonizing the results gathered from studies of muscle enzymes with those derived from investigations of intact muscle.

Hevesy and Rebbe (114) showed that radioactive inorganic phosphate injected into frogs has entered the creatine phosphate fraction after 3 hr. at 2°C. to an extent of 49 per cent and at 21°C. to an extent of 78 per cent.

Korzybski and Parnas (145) injected radioactive phosphate into mammals and found that an equal distribution of radioactive phosphate between inorganic phosphate, creatine phosphate, and adenylypyrophosphate in the muscle had been reached 60 min. after the injection. Hevesy and collaborators (113) find that the plasma phosphorus enters the muscles very slowly. After 3 hr. the plasma still contains twelve times more radioactivity per milligram of inorganic phosphorus than the inorganic phosphorus fraction in the muscles. The radioactive phosphorus which has entered the muscle appears very soon in the phosphorus of the esters. This may explain the result of some recent experiments by Sacks (253), which disagree with the investigations of Hevesy and Rebbe and those of Korzybski and Parnas. Sacks found that 2 hr. after the injection of

radioactive phosphorus the creatine phosphate and adenylypyrophosphate fractions contain only 10 to 15 per cent of radioactive phosphate (10 to 15 per cent saturation).

Sacks analyzes the muscles 2 hr. after the injection of radioactive phosphorus, using very strong preparations. At that time the difference in radioactivity per mg. of phosphorus between plasma and muscle must be very great (considerably greater than 12:1) and a small amount of blood in the muscle analyzed would increase the radioactivity in the so-called inorganic phosphorus fraction in muscle very much.³⁸ This might be the whole explanation of the disagreement between the results of Hevesy and of Korzybski and Parnas on the one side and those of Sacks on the other. It is at least obvious that the possibility of "plasma contamination" must be mentioned and accounted for.

If the strong radioactivity of the inorganic phosphorus is not due to "blood contamination," the question arises whether the radioactive phosphate is localized in the connective tissue or inside the muscle cell. It is at least obvious that Sacks is not justified in rejecting the Embden-Meyerhof scheme on the basis of his experiments.

XI. CONNECTIONS BETWEEN BIOLOGY AND PHYSICS

The aim of this review has been not only to collect and coördinate knowledge from very different fields, like animal physiology, microbiology, enzyme chemistry, organic and physical chemistry, but also to interpret all the fundamental biological phenomena from a dynamic point of view.

The recent revolutionary progress in our understanding of the coupling between oxidations and phosphorylations has led to new problems, particularly in the field of physical chemistry. It has been pointed out previously in this review that there exists a great lack of physical data for the different states of inorganic phosphate (ortho-, meta-, pyro-, and tri-phosphate). It would be of great importance for biologists to have exact physical data for pyrophosphate linkages, especially in the light of the recent discoveries of the formation of the latter from carboxyl phosphates. Furthermore, certain physical methods (for instance, Raman spectra) might be used to detect and estimate the very labile carbonyl phosphates which are supposed to be the reactive form in which carbonyl groups are oxidized in biological systems. The branch of physical chemistry which deals with the structure of chemical compounds can undoubtedly be of the greatest value for biological chemistry. The concept of resonance makes it possible to account qualitatively for a number of fundamental biochemical reactions. Quantitative data for the thermodynamic stability of different

³⁸ A muscle frozen during or just after a contraction contains, of course, much more blood than a resting muscle.

types of ester linkages could probably be obtained from experimental work in the field of structural chemistry.

In any case it would be a very great advantage for biological sciences if physicists and physical chemists would pay more attention to the fundamental well-defined chemical reactions which are the driving forces behind the various manifestations of life.

XII. SUMMARY

The fundamental reactions, oxidation and reduction, are defined as the removal of electrons (ϵ) and uptake of electrons, respectively. In organic systems oxidations are usually accompanied by proton (H^+) liberation, and reduction by proton uptake. Oxidations are endergonic ($+\Delta F$) and reductions are exergonic ($-\Delta F$) in redox systems which have positive normal potentials. In redox systems with a negative normal potential, oxidations are exergonic and reductions endergonic. Among the metabolites, fatty acids (paraffins, olefins) represent rather positive systems [E'_0 (pH 7) slightly positive], while sugars and sugar acids (carbonyl (H_2O), carboxyl) represent strongly negative (reducing) systems.

Biological systems transfer electrons from negative redox systems (electron donors) to positive systems (electron acceptors); this electron transfer takes place in cellular respiration or fermentation.

In respiration the electron acceptor is obtained from the environment in the form of oxygen; in fermentations the electron acceptor has to be formed from the electron (hydrogen) donor.

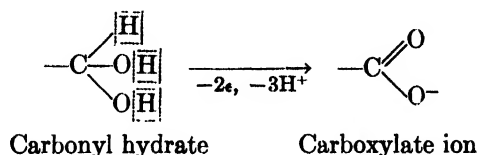
The transfer of electrons from donor to acceptor never takes place directly but always stepwise through one or several transfer systems. The larger the difference between the normal redox potentials (E'_0) of electron donor and electron acceptor, the more electron-transfer systems are interposed.

The nature of the electron-transfer systems is known; most essential in the electron-transfer system of fermentations is a pyridine compound in combination with a specific protein catalyst. α -Keto acids do not react with pyridine compounds but with thiazole compounds. In respiration, alloxazine and iron porphyrin compounds are interposed in addition to pyridine or thiazole compounds.

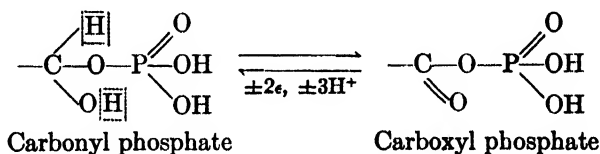
Electron acceptors (double bonds) represent reactive structures, exhibiting paramagnetic properties. Electron donors, i.e., ordinary metabolites, show no paramagnetic properties; these compounds have to be activated by some specific protein catalyst. The nature of the action of specific, catalytically active proteins is not known, but there is some evidence that they decrease the potential barrier between the valence-saturated compound and the reactive free radical (semiquinone).

A number of electron donors (metabolites) cannot be oxidized unless orthophosphate is present; this is the case with triose phosphate and pyruvic acid. These oxidations were said to be "coupled" with a phosphate uptake, since the phosphate was found in organic compounds after the oxidation. The rôle of phosphate in the oxidation of carbonyl groups has been revealed recently. It was generally believed that oxidation of

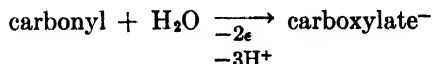
—C(=O)H groups to —C(=O)O⁻ requires the uptake of water:



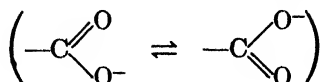
Modern enzyme studies have shown that not water but phosphate is taken up in the biological carbonyl oxidations:



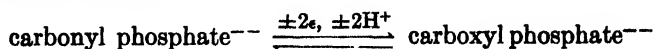
Arsenate can replace phosphate. Two types of carboxyl phosphate have been discovered: glyceryl phosphate (isolated as 1,3-diphosphoglyceric acid) and acetyl phosphate. Both show the same absorption line ($m\mu$ 217) as acetic acid anhydride (acetyl acetate). The thermodynamic consequences of the new reactions are far reaching. Thermal data show that the reaction



involves a large decrease in free energy, i.e., a large increase in stability, due to the formation of the resonating carboxylate structure

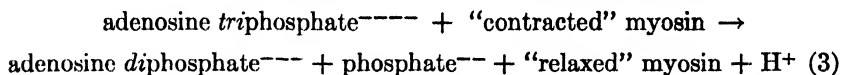
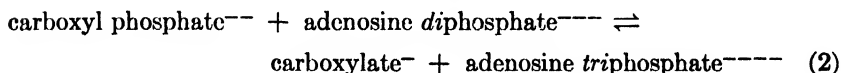


The ΔF of the reaction



is small, since this reaction has been shown to be easily reversed. Thus the replacement of water by phosphate in the carbonyl oxidation means

that the main part of the free energy of the carbonyl group is not released but is kept in certain acid anhydrides and transferred to structures where it has a chance to be transformed into mechanical work. It has been shown that the phosphate of carboxyl phosphates can be directly transferred by a specific enzyme to form pyrophosphate compounds (adenosine polyphosphates); this reaction is easily reversed, i.e., ΔF is small. The dephosphorylation of the pyrophosphate linkages is known to be one of the most strongly exergonic biochemical reactions. There is increasing evidence that the dephosphorylation of the pyrophosphate compound is linked with the relaxation of contracted myosin, i.e., the recharging of the discharged contractile system. The discharge may "regulate" the metabolism of sugar (carbonyl) in the following manner:



Since reactions 1 and 2 are reversible reactions, the enzymatic oxidation of carbonyl to carboxylate cannot proceed unless the irreversible reaction 3 takes place. Thus, the amount of "contracted" myosin regulates the oxidation of carbonyl compounds.

The replacement of water by phosphate has also been shown in another type of reaction: namely, the breakdown of polyhexoses (starch and glycogen). Certain enzymes in the digestive tract catalyze the splitting of glycoside linkages by water. These enzymes (diastases, amylases) hydrolyze starch and glycogen to glucose. The reaction is practically irreversible, i.e., addition of glucose to this enzyme does not yield any detectable amount of polyhexoses.

In the tissues glycogen is broken down not by a hydrolysis but by a phosphorolysis, i.e., phosphate breaks the glycoside linkages of the polyhexose, and 1-phosphoglucose is formed as a primary product. The reaction



is very easily reversed; addition of 1-phosphoglucose to the specific enzyme (phosphorylase) immediately yields large amounts of polyhexose.

Thus, the replacement of water by phosphate converts strongly exergonic reactions like oxidation or the splitting of glycoside linkages, to nearly energy-neutral reactions.

We are now able to perceive clearly that the "expensive" steps in the

biological reduction of acids to aldehydes or in the biological polymerization of monohexoses to polyhexoses are not the reduction proper nor the polymerization but the formation of energy-rich phosphorylated precursors (i.e., carboxyl phosphate and 1-phosphoglucose, respectively).

The great progress made in the last three or four years is mainly attributable to the isolation and identification of these precursors and to the separation and isolation of the specific protein catalysts.

In writing this survey I have had valuable support and help from many quarters. It is a pleasure for me to thank Prof. Linus Pauling and Dr. E. R. Buchman, Gates and Crellin Laboratories of Chemistry, California Institute of Technology, for their encouragement and support in the publication of this material, part of which has been delivered in seminars at the California Institute of Technology.

Dr. C. D. Coryell, Department of Chemistry, University of California, Los Angeles, has been so kind as to undertake an examination of this paper, in particular of the physical-chemical sections. I am very thankful to Dr. Coryell, not only for his careful examination but also for his brilliant suggestions, which have been of much importance for my own comprehension of some of the most fundamental problems in this review.

My thanks are due to Prof. Henry Borsook and Prof. Hugh M. Huffman, Department of Biochemistry, California Institute of Technology, for their kind help in thermodynamic questions and for access to new thermal data. I also wish to thank Prof. C. B. van Niel, Hopkins Marine Station, Pacific Grove, for very valuable advice, discussions, and suggestions concerning microbiological problems.

Finally, I wish to thank Prof. C. F. Cori, Dr. G. T. Cori, and Mr. S. P. Colowick, Medical School, Washington University, St. Louis, for their valuable and helpful criticism of the manuscript.

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As early as the thirteenth century Marco Polo¹ wrote about one section of western China:

Throughout all the mountainous parts of it the most excellent kind of rhubarb is produced in large quantities, and the merchants who come to buy it convey it to all parts of the world. It is a fact that when they take that road, they cannot venture amongst the mountains with any beasts of burden excepting those accustomed to the country, on account of a poisonous plant growing there, which, if eaten by them, has the effect of causing the hoofs of the animals to drop off. Those of the country, however, being aware of its dangerous quality, take care to avoid it.

About six centuries later, stockmen in some sections of the Great Plains described symptoms similar to those related by Marco Polo. Prior to the settlement of the western part of the Great Plains area, Madison (119), a surgeon in the United States Army, described cases of sickness in cavalry horses when they ate native vegetation along the Missouri river near the present boundary between South Dakota and Nebraska.

When stockmen moved into some regions they were unable to graze livestock without losses; later, farmers had the same experience with the grains and forages produced on their farms. At first the reports of a strange malady of livestock received little attention, but after many years the numerous pleas for aid were recognized and several agricultural experiment stations undertook to determine the cause.

The investigations which culminated in the discovery of selenium as the etiological agent were largely carried out by Dr. Kurt W. Franke, who began studies at the South Dakota Agricultural Experiment Station in the fall of 1928. Franke observed the symptoms of poisoning in farm animals and obtained numerous feed samples from those regions where the poisoning was more severe. When he tested these feeds with laboratory animals, he soon showed that the feeds contained a poison and that the observed symptoms of poisoning were not due to the water, as many farmers and stockmen believed. All grains and forages grown on some farms were extremely toxic.

When the problem was called to the attention of the United States Department of Agriculture in 1932, several bureaus became actively interested. Because the symptoms of poisoning indicated a metallic poison, a systematic search for trace elements was made of a sample of grain which Franke had found toxic by bioassay. This led to Robinson's (156) discovery, in 1933, of the presence of selenium.

All of the cereal samples which Franke (57) found to be toxic by bioassay with rats contained selenium. The protein of the cereal grains carried most of the poison (58), and the selenium was confined chiefly to the

¹ *The Travels of Marco Polo*, edited by Manuel Komroff, Chapter 43, page 81. Boni and Liveright, New York (1926).

protein. Selenium was detected in the soil of all known regions where toxic grain grew, but there was still a possibility of the presence of other poisons. In some seleniferous plants Beath, Eppson, and Gilbert (9) found molybdenum and tellurium, and Byers (28) reported chromium, vanadium, and arsenic in a seleniferous soil. The first biological evidence to indicate that selenium was the sole causative agent was the production, by the addition of sodium selenate and sodium selenite to an otherwise normal diet, of symptoms in the rat (Franke and Potter (68)) which appeared identical with those produced by the natural toxicant. By injection of selenium salts into hens' eggs, Franke, Moxon, Poley, and Tully (62) caused the development of monstrosities similar to those previously found in chick embryos (71) from eggs laid by hens which had been fed toxic grains. Franke and Painter (64) removed nearly all of the selenium from a hydrolysate of a seleniferous protein and then found the hydrolysate to be non-toxic when fed.

B. THE TOXICITY OF SELENIUM

1. *General*

Franke, Rice, Johnson, and Schoening (70) have described the symptoms of the chronic selenium poisoning,—usually called “alkali disease”,—of farm animals. All animals lose weight and appear emaciated. There is loss of hair from the mane and tail of horses, from the switch of cattle, and from the body of swine. In severe cases there is a discontinuity in the growth of the hoof, which is followed by a sloughing-off of the old hoof. Post-mortem examinations reveal severe lesions at the joints, which probably explain the lameness. Chick embryos are deformed, especially in the upper beak, so that eggs frequently fail to hatch. This chronic type of poisoning rarely causes death except in young animals, but the economic losses to farmers are large.

Symptoms which appear to be due to acute selenium poisoning were described by Draize and Beath (41). This toxicosis is sometimes called “blind staggers,” because the animals lose control of their voluntary muscles. There are reports of enormous losses of livestock from a single feeding of toxic vegetation. Although the reviewer is aware that some plants contain large quantities of selenium, observations of the effects of feeding seleniferous feeds to laboratory animals makes it seem incredible that an animal would voluntarily ingest enough at one time to develop acute poisoning.

Presumably the chronic type of poisoning is the result of prolonged ingestion of forages and grains containing from 10 to 30 p.p.m.² of selenium.

² p.p.m. = parts per million.

These are levels often found in grasses (124) and in farm crops in seleniferous areas. A few plants, however (part I, C), may accumulate several thousand p.p.m. of selenium. In some sections in and around the Rocky Mountains, several of these range plants are eaten by livestock. In addition to selenium, some of these plants contain organic poisons which may contribute to the acute symptoms sometimes noted in range stock.

Of the numerous reports on the toxicosis produced by seleniferous diets in experimental animals, Franke and coworkers (57, 64, 66, 67, 68, 69), Munsell, Devaney, and Kennedy (138), and others have studied the effects on the rat. Franke (57) found that diets which contained from 70 to 82 per cent of toxic grains killed about 70 per cent of young rats by the sixtieth day of ingestion. Analysis (66) showed that most of these grains contained from 25 to 30 p.p.m. of selenium. In general, the animals failed to grow, restricted their food intake, frequently became jaundiced, and after several weeks developed anemia (67, 68). Autopsy revealed hemorrhages, lesions at the joints, necrotic livers if the rats were on a seleniferous diet for an extended period, and, frequently, enlarged spleens. Death was often caused by internal hemorrhage.

The results of feeding seleniferous diets to poultry have been reported in numerous papers by Franke and Tully (71, 178) and Poley, Moxon, and Franke (149).

A few papers have appeared on the accumulation, detoxification, and elimination of selenium (138, 130, 136, 165). Large quantities of selenium are found in the liver, kidneys, and spleen with lesser amounts in other organs and tissues. Selenium is eliminated primarily through the kidneys, but some may be exhaled.

2. Different sources of selenium

Selenium from different sources is not equally toxic. When the results of feeding seleniferous diets to rats were summarized, Franke and Painter (66) found the order of toxicity of selenium from several sources to be as follows: wheat > corn > barley > selenate > selenite > selenide > metallic selenium. There was little difference in the toxicity of selenium in cereals, but these were definitely more toxic than inorganic selenium salts. From the data of Smith, Stohlman, and Lillie (164) it would appear that selenite is more toxic than selenate. Since the chemical evidence indicates that selenium in plants is in organic forms (part IV), the toxicity of diselenodiacetic acid, selenodiacetic acid, β, β' -diselenodipropionic acid, β -selenodipropionic acid, dibenzyl diselenide, β -seleninopropionic acid, and *n*-propylseleninic acid was compared with that of selenite by Moxon, Anderson, and Painter (130). None of the organic compounds was as toxic as selenite, but they produced similar symptoms of poisoning.

Evidence that selenium in cereals is more toxic than selenite may be found in the data of Schoening (159) and of Miller and Schoening (125), in addition to the results of Franke and Painter (66). Although corn, with 10 p.p.m. of selenium, produced symptoms of poisoning in swine, much larger quantities of selenite were tolerated.

Not all species react similarly to selenium. It has been observed on farms and in the laboratory that adult animals are less susceptible to selenium poisoning than younger animals. Trelease and Trelease (175) presented evidence that some insects are so resistant to the poisonous action of selenium that they thrive on food sources which would be fatal to most animals.

Dudley (46) has found selenium oxychloride to be toxic when applied to the skin. As little as 0.01 ml. caused death in rabbits and third-degree burns when applied to the skin of man. Concentrations of hydrogen selenide in the air as low as 0.02 mg. per liter killed guinea pigs within 25 days (49) on exposure for 60 min per day.

Franke and Moxon (61) have compared the toxicity of orally ingested selenium as Na_2SeO_3 and Na_2SeO_4 with that of arsenic as Na_2HAsO_3 , of molybdenum as $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, of tellurium as Na_2TeO_3 , and of vanadium as NaVO_3 . When compared at equal weights of the elements, none of the salts was as toxic as selenium. Martin (122) found that tellurium was less toxic than selenium to animals and plants.

3. Injection of lethal doses of selenium

Several papers have appeared on the results of the injection of selenium compounds. These are summarized in table 1.

The results from different laboratories are not exactly comparable, because the terms "minimal lethal dose" or "minimal fatal dose" are often not rigidly defined. In spite of this, the variations in the results on the same species with the same sources of selenium cannot be explained by the different methods of injection, by the difference in the percentage of mortality, or by the time allowed to elapse before death. Regardless of these differences, the data show more clearly than the results of oral feeding that some species of animals are more resistant to selenium poisoning than others and that there is a great difference in the toxicity of different selenium compounds. The high resistance of cattle and swine to selenium is surprising, because on farms both species often show marked symptoms of poisoning. It seems doubtful if the drench method is a true measure of tolerance to selenium in cattle, because absorption from the rumen is slow and in the presence of organic material some reduction of selenite would be likely to occur.

Shortly after the injection of lethal doses of selenium (60), animals

exhale an odoriferous (garlic-like) compound which Hofmeister (89) reports to be methyl selenide; the evidence for its being methyl selenide is

TABLE 1
Toxicity of single doses of selenium

SOURCE OF SELENIUM	ANIMAL USED	FATAL DOSE IN MILLI-GRAMS OF SELENIUM PER KILO-GRAM OF BODY WEIGHT	NUMBER OF ANIMALS	METHOD OF ADMINISTRATION	OBSERVERS	REFERENCE
Na_2SeO_3	Rat	5 7			Muehlbeyer and Schrenk	(137)
Na_2SeO_3	Rat	3 25 3 50	155	Intraperitoneal injection	Franke and Moxon	(60)
Na_2SeO_3	Rat	3 0	45	Intravenous injection	Smith, Stohlman, and Lillie	(164)
Na_2SeO_3	Rabbit	1 5	9	Intravenous injection	Smith, Stohlman, and Lillie	(164)
Na_2SeO_3	Rabbit	0 9			Muehlbeyer and Schrenk	(137)
Na_2SeO_3	Horse	<4 4	5	Stomach tube	Miller and Williams	(126)
Na_2SeO_3	Mule	3 3±	3	Stomach tube	Miller and Williams	
Na_2SeO_3	Cow	11 0±	5	Drench	Miller and Williams	
Na_2SeO_3	Pig	15 0±	5	Drench	Miller and Williams	
Na_2SeO_4	Rat	4.3			Muehlbeyer and Schrenk	(137)
Na_2SeO_4	Rat	5 25-5.75	90	Intraperitoneal injection	Franke and Moxon	(60)
Na_2SeO_4	Rat	3.0	37	Intravenous injection	Smith, Stohlman, and Lillie	(164)
Na_2SeO_4	Rabbit	2.0-2.5	16	Intravenous injection	Smith, Stohlman, and Lillie	
Colloidal selenium	Rat	6 0			Muehlbeyer and Schrenk	(137)
Organic selenium compounds.	Rat	20-40		Intraperitoneal injection	Moxon, Anderson, and Painter	(130)
Organic selenium compounds.	Rat	>25		Intraperitoneal injection	Moxon	(129)
<i>d,l</i> -Seleno-cystine	Rat	4.0	65	Intraperitoneal injection	Moxon	(129)

far from convincing, as Schultz and Lewis (160) point out. The reviewer cannot distinguish the odor from that of hydrogen selenide. Respiration

becomes increasingly difficult and the animals die gasping for breath. In some cases there is complete anesthesia just before death, but in other cases there is a convulsive struggle. Loss of fluid from the blood into the abdominal and thoracic cavities may cause the hemoglobin level to reach 30 g. per 100 ml. of blood (68).

Franke and Moxon (60) injected the same salts of arsenic, molybdenum, tellurium, and vanadium as were fed orally (61). At equal weights of the element, the salts decreased in toxicity in the following order: tellurite, selenite, vanadate, arsenite, selenate, arsenate, tellurate, and molybdate. There was a much greater difference between the toxicity of tellurite and tellurate and of arsenite and arsenate, than between that of selenite and selenate. Molybdenum, which Beath, Eppson, and Gilbert (9) found to be taken up by plants in quantities which produced symptoms of poisoning in animals, was non-toxic at the levels injected (160 mg. per kilogram of body weight) and when fed (61).

4. Selenium in human nutrition

Several papers on the possibility of selenium poisoning in humans have appeared. The discovery of the presence of traces to as high as 1 p.p.m. of selenium in the urine of the majority of people living in highly seleniferous areas at first appeared alarming. From the data of the first survey, Smith, Franke, and Westfall (163) found no symptoms which could be considered pathognomonic of selenium poisoning in man; later, Smith and Westfall (166) believed they had evidence that selenium caused gastric or intestinal dysfunction, and possibly hepatic dysfunction, in some sections. These findings are not surprising where locally grown food supplies a large proportion of the diet. Meat, milk, eggs, and vegetables may contain considerable quantities of selenium when produced on farms in the seleniferous areas. Manville (120) emphasizes the potential danger of selenium-bearing foods to public health.

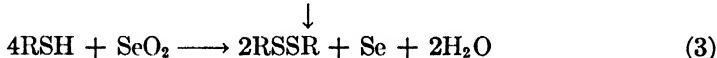
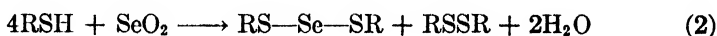
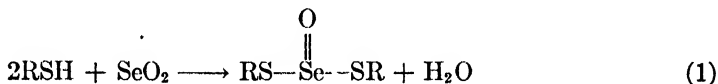
Dudley (47) stresses the potential danger of selenium injury in industrial operations, particularly in copper refining. He describes, as did Hamilton (87), symptoms of poisoning presumably caused by the inhalation of hydrogen selenide, selenium dioxide, and other selenium compounds. The concentration of selenium in the urine of industrial workers showing marked pathological symptoms (44) was less than one-tenth of that of many human urines (163) taken in seleniferous areas. Elimination of inorganic selenium taken into the lungs may be more rapid and follow other pathways than organic selenium taken orally, but these results are difficult to explain in view of the recorded evidence that selenium in food-stuffs (66) is more toxic than inorganic selenium. Hydrogen selenide is volatile and it may be very toxic when inhaled. Franke and Potter (68) found sodium selenide much less toxic to rats than sodium selenate or

sodium selenite when fed, but it is likely that some oxidation of selenide to elemental selenium occurred before absorption.

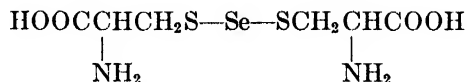
In this connection one might describe the symptoms observed by the reviewer from a single inhalation of hydrogen selenide in high concentration, which passed about 4 in. along the nasal passage. As the vapors traversed the nasal passages there was a metallic sensation, somewhat like that produced by a silver nitrate spray. After a brief sensation of intoxication, no ill effects were felt for about 4 hr. Then a copious discharge of mucous from the nasal passages began. This persisted, with violent sneezing similar to the symptoms of a severe head cold, for 3 or 4 days. No ill effects were noted later. The author has never had "selenium breath," which is rumored to result from working with selenium compounds.

5. Action of selenium in the animal body

Little is known of the mechanism of selenium poisoning. It inhibits carbon dioxide production during yeast fermentation (132), as well as the oxygen uptake of yeast cells (152). Selenite readily oxidizes sulfhydryl compounds, forming disulfide and an unstable RS—Se—SR compound. With sulfhydryl compounds the reaction may take three courses:



The reviewer (unpublished work) has obtained an amino acid from the action of selenite on cysteine; this acid is thought to be



In every preparation isolated, the mole S:Se ratio was slightly greater than 2, owing to the presence of some cystine. Separation of compounds of the type RS—Se—SR from disulfides is difficult, because of the instability of the former. Metallic selenium separates from solution,—but more rapidly from basic than from acidic solutions,—to form the disulfide. Bersin (18), who prepared the compound $\text{HOOCCH}_2\text{S}-\text{Se}-\text{SCH}_2\text{COOH}$ from thioglycolic acid and selenite, believes that a similar unstable compound forms with glutathione. Seleninic acids will also reduce sulfhydryl compounds to disulfides (unpublished work), but no addition compound has been isolated.

It does not seem improbable that selenite or seleninic acids inhibit certain enzymatic reactions dependent upon reversible sulfhydryl \rightleftharpoons disulfide changes and those systems which require the presence of free sulfhydryl groups, i.e., succinic dehydrogenases. In addition to the chemical studies, Dubois, Rhian, and Moxon (43) find that glutathione when injected will protect rats from doses of selenite which will normally cause death. Selenium in its natural forms in plants must react in a manner different from selenite, because the chemical evidence (part IV) indicates that it is in the reduced form.

The ability of some proteins, when fed at high levels, to counteract the chronic symptoms of selenium poisoning in rats was observed by Moxon and has been further studied by Gortner (84a) and by Lewis, Schultz, and Gortner (118a). This protective action of some proteins cannot at present be ascribed to any particular amino acid of the proteins. Not all "complete proteins" are effective. In some cases methionine,—but not cystine, —supplements (118a) to diets were as effective as high protein, but with other proteins (84a) methionine was not beneficial.

Recently Moxon and Dubois (131) and Dubois, Moxon, and Olson (42) have shown that small amounts of arsenic will alleviate the toxicity of selenium to animals. The exact rôle of arsenic seems very obscure.

C. SELENIUM IN SOILS AND IN PLANTS

Byers (29, 30, 33), Beath and coworkers (8, 11, 12, 111), and Moxon and coworkers (133, 134) have shown that selenium occurs in rocks and soils from the Niobrara, Pierre, Steele, Benton, and other geological formations. The most complete studies of certain formations, those by Moxon *et al.* (133, 134), have shown that the selenium content varies in certain members of these formations, so that highly seleniferous areas may be predicted from the geological formation. It is generally stated that selenium was deposited during the Cretaceous period, but Beath and coworkers (11, 12, 111) find highly selenized soils geologically much older than Cretaceous. After studying the seleniferous soils of Hawaii, Byers, Williams, and Lakin (34) suggested that the selenium in soils is of volcanic origin. Selenium in volcanic emanations may be a primary source of selenium in soils, but the work of Moxon, Olson, and Searight (133) indicates that the selenium in soils of the continental United States is of marine origin.

The surveys conducted by Byers (29, 30, 33) have revealed seleniferous areas extending west from the western Dakotas, Nebraska, Kansas, and Oklahoma to the coast states. One must not gain the impression that the entire area, most of which is marginal agriculturally, is seleniferous. There are, however, sections which are producing seleniferous crops, or are

potential producers, because there is selenium in the soil. As many agricultural sections in this country are underlaid with seleniferous formations which have been covered with glacial drift, it was concluded that glaciated areas were free from seleniferous plants. Recently, Byers and Lakin (32) pointed out a large area in western Canada and in the northwestern United States which was glaciated but which produces highly seleniferous plants. Analysis by Robinson (157) of crops from various parts of the world showed that seleniferous areas are widespread over the surface of the earth.

Rivers and ground waters rarely contain detectable amounts of selenium. Waters of the Colorado river and its tributaries are free from selenium above diversion points (33, 183), but from the points where the drainage from irrigated lands is put back into the river the selenium concentration increases.

Selenium is found in much the same mineral deposits (170) and soil formations as those where sulfur abounds. In this connection the presence of selenium in deep sea deposits (133, 182), in sea water (84), and in meteorites (31, 170) is of interest.

The amount of selenium absorbed by plants is dependent more on the availability of the compounds of selenium than on the selenium content of the soil. The forms of selenium generally considered to be present in soils are as follows: (1) elemental, (2) pyritic or selenide, (3) selenite, (4) selenate, and (5) organic. Very little is in the elemental form (33). In sulfide ores, usually iron pyrites (161), selenium is often present in high concentrations, but the selenium in pyritic concretions of soils is not an important direct source of selenium in plants. Since (1) much of the selenium in seleniferous soils is very insoluble, (2) seleniferous soils are highly ferruginous, and (3) very insoluble compounds of selenium form with selenite and ferric iron (170), Williams and Byers (184) have concluded that a major portion of the selenium in many soils is present as an insoluble basic iron selenite. When they (184) precipitated compounds of ferric selenite from dilute solutions of ferric chloride and sodium selenite, the composition varied with the Se:Fe ratio. Some of their preparations had a composition which could be approximately defined by the formula $\text{Fe}_2(\text{OH})_4\text{SeO}_3$, but in all of their work the Se:Fe ratios were many times greater than the Se:Fe ratios in soils. As supporting evidence for the presence of selenate (probably as CaSeO_4), Williams and Byers (184) found that the water-soluble selenium in soils was reduced to elemental selenium by the same methods which reduce selenate. Olson and Moxon (141) presented data to indicate that a considerable quantity of organic selenium occurs in soils. Humus may contain approximately 40 per cent of the selenium in some soils.

Before selenium can be taken up by plants, it must be present in available

soil forms. There is a great difference in the availability of different compounds. Moxon, Olson, and Searight (133) grew plants on a non-seleniferous soil to which selenium was added at the rate of 2 p.p.m. from the following sources: Na_2SeO_4 , CaSeO_4 , Na_2SeO_3 , $\text{Fe}(\text{OH})\text{SeO}_3$ (approximate composition), FeSe , and organic selenium (a water extract of *Astragalus*). Selenium was taken up from selenates in large amounts and from selenites in moderate amounts; from iron selenide and from organic selenium there was no absorption of selenium by some cereals and only a very small amount by *Astragalus*. The results with selenites did not support Byers' contention that selenium is relatively non-available in basic ferric selenite. He accounted for the fact that plants growing on moderately to highly seleniferous soils in Hawaii and Puerto Rico have not been found to contain over 3 p.p.m. of selenium (112), by assuming that the selenium is in the form of an exceedingly non-available basic ferric selenite. Obviously the composition of a soil is an important factor in the availability of different compounds of selenium. Selenite may be firmly bound in soils, as Franke and Painter (65) were unable to electro-dialyze all of the selenite added to a suspension of a seleniferous soil. Apparently sufficient time was not allowed by Moxon, Olson, and Searight (133) for bacterial decomposition of the organic selenium compounds, because Beath *et al.* (10) have found these forms of selenium available to plants.

Presumably areas of high rainfall are relatively free of danger from poisonous quantities of selenium in plants, even though the soils are seleniferous. The percolating action of water would remove the more available soluble selenium salts and soluble organic compounds. The selenium would ultimately find its way to the sea. Most soils producing seleniferous crops are immature because they have weathered slowly.

Concomitant with the available selenium in soils is the variable capacity of different plants for absorbing selenium. Cereal grains are moderate absorbers of selenium (124) but rarely contain more than 30 p.p.m. Of the plants in the seleniferous areas, some native grasses absorb the least amount of selenium. A few plants,—notably some species of the genera *Stanleya*, *Oenopsis*, *Astragalus*, and *Xylorrhiza*,—often accumulate several thousand parts of selenium per million. The selenium contents of a group of plants and of the soils upon which the plants were growing are shown in table 2. These results were taken from tables in numerous publications from the laboratories of Franke and Moxon, of Byers, and of Beath. The soils are in the seleniferous areas of the continental United States and range from what may be considered highly seleniferous to mildly seleniferous.

The data show the wide variations in the absorption of selenium by different plants and the variations which occur between the selenium content of the plant and that of the soil upon which the plant was growing.

Presumably the differences were due primarily to different selenium compounds in the soil. The last three species, commonly called indicator plants, have the capacity of absorbing selenium from forms only slightly available to the other plants. Beath, Eppson, and Gilbert (10) found that *Astragalus* would absorb selenium from soils artificially selenized by elemental selenium.

TABLE 2
Selenium content of plants and of soils

VEGETATION	SELENIUM CONTENT							
	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant
	p p.m.	p p.m.	p p.m.	p p.m.	p p.m.	p p.m.	p p.m.	p p.m.
Wheat	12 0	40	3 5	40	3 1	25	1.5	4
Corn	3 5	10	3 1	23	2 0	1	0 3	0
Barley	3.1	22	2 5	10	2 0	12	1 0	1
Alfalfa	2 0	12	1 5	60	0 7	1	0 5	25
Sweet clover	9 0	50	2 0	3	1 5	25	1.5	1
Western wheat grass	13 0	10	5 5	30	0 7	12	0 5	25
Blue gramma	6 0	4	3 5	2	2.0	2	0 2	0
Little bluestem	9 0	1	4.0	5	3 5	1	1 0	0 5
Mixed native grasses	20 4	47	6.0	3	3 0	1	0.5	3.5
Russian thistle	3.0	3	2.0	12	1.0	40	0 7	3
Sunflower	3 0	12	0 7	7	0 7	4	0.6	1
Wreath aster	8 0	130	4 0	7	1.0	210	0 3	1
Woody aster	3 5	5390	2 0	200	0 7	120	0.3	6
<i>Stanleya pinnata</i>	20.4	1252	5 0	470	4 0	1070	1.0	20
<i>Oenopsis condensata</i>	20 4	3250	8 1	664	3 5	9120	1 5	850
<i>Astragalus racemosus</i>	27 0	1160	10 0	1690	5 0	5560	2.5	60
<i>Astragalus racemosus</i>	21 0	4100	6.1	2700	5.0	690	0.7	920
<i>Astragalus bisulcatus</i>	20.4	2590	3.0	170	2 0	2120	0 8	3030
<i>Astragalus bisulcatus</i>	8 1	5330	2 5	590	1.5	3140	0.7	100
<i>Astragalus pectinatus</i>	8 0	1330	3 5	4000	2.5	2270	1 5	3890
<i>Astragalus pectinatus</i>	5 0	1980	3.0	2590	2 0	2120	0.2	840

Recently Beath, Eppson, and Gilbert (12) have used the occurrence of selenophilic indicator plants to locate seleniferous soils throughout the country. Sufficient data have been presented to warrant the conclusion that these indicator plants abound on most seleniferous soils. Selenite and selenate (93) are toxic to many plants, but it is doubtful if the concentrations in soils are great enough to explain the absence of these plants on some seleniferous soils. Since Trelease and Trelease (176) find selenium to be a stimulating and possibly an essential element in the metabolism of

some species of *Astragalus*, it may be that the indicator plants compete more successfully with other plants when growing on seleniferous soils.

Indicator plants are potential accumulators of available selenium in soils. Beath *et al.* (10) have shown that, whereas only indicator plants absorb large quantities of selenium from raw shales, the selenium from these plants is readily taken up by other plants, either as soluble organic compounds or after bacterial decomposition and oxidation to inorganic salts. Thus the soils are enriched with available selenium through countless cycles of growth and decay by these converter plants.

D. SELENIUM AND SULFUR IN PLANTS

Because of the similar chemical properties of selenium and sulfur, considerable speculation has appeared in the literature as to whether or not selenium compounds analogous to those of sulfur occur naturally in

TABLE 3
Selenium and sulfur in plants

CROP	SULFUR CONTENT	SELENIUM CONTENT
	<i>per cent</i>	<i>per cent</i>
Cabbage	2.99	0.0520
Black mustard . .	1.97	0.0470
Flax	1.32	0.0358
Vetch	0.77	0.0150
Wheat	0.84	0.0225
Soybean..	0.51	0.0140
Corn	0.42	0.0075

seleniferous plants. Aside from the analogous chemical behavior, some studies of plant metabolism have indicated a relationship between these elements.

As early as 1880, Cameron (35) found that plants would absorb selenium and suggested that it might replace sulfur. Hurd-Karrer (94, 96) has found that, in general, plants which absorb large quantities of sulfur absorb large quantities of selenium. A few analyses of young plants reported by Hurd-Karrer (96) are shown in table 3.

From the enormous quantities of selenium absorbed by indicator plants, it would seem that the absorption of selenium is out of proportion to the available sulfur and selenium in the soil, but when grown under experimental conditions *Astragalus bisulcatus*, a wild legume, absorbed selenium and sulfur in a ratio consistent with that found in other plants of the same genus.

The discovery that sulfate or elemental sulfur (93) would diminish selenium injury to plants and reduce the quantity of selenium absorbed

was received with considerable enthusiasm. Hurd-Karrer's (93, 94) results indicated a definite antagonism between the two elements, because the absorption and toxicity was dependent upon the S:Se ratio in the nutrient solution. Martin (121) found less inhibition by sulfur of the toxicity of selenium to plants than did Hurd-Karrer in artificially selenized soils, and Franke and Painter (65) were unable to reduce, by the application of sulfur, the absorption of selenium in crops grown on naturally seleniferous soils. Sulfur was without effect when the source of the selenium in the soil was seleniferous *Astragalus* (10). Most seleniferous soils are abundant in gypsum. Olson and Moxon (141) believe that the forms of selenium in soils have a much greater influence on the absorption of selenium than does the sulfate content.

The divergent results from several laboratories find explanation in later studies by Hurd-Karrer (95, 97), in which she shows that injury by selenate and absorption of selenium decreased progressively with increased sulfate concentrations, but that, with selenite, sulfate was effective over limited changes in concentration. High sulfate concentration did not reduce the selenium in plant tops when selenite was the source of selenium. Thus with analogous compounds,—i.e., selenate and sulfate,—there is a definite relationship in the absorption which depends upon the concentrations. A preferential absorption of sulfur was evident.

In the cereal grains Franke and Painter (63, 145) found most of the selenium in the protein, as is the case with sulfur. The mole S:Se ratios (145) in the whole grain and protein were generally close.

Many of the chemical properties of selenium in plants (part IV) are similar to those of sulfur. If selenium analogs of sulfur compounds are present, the number in most plants is small and confined to a few types. The sulfur compounds in higher plants are of the following types: RCNS (in glucosides), RSR (vinyl and allyl sulfides and in methionine), RSCH₂SR (the cysteine thioacetal of formaldehyde in djenkolic acid), RSH and RSSR (cysteine, cystine, and glutathione). All of these are straight-chain systems. A few other compounds,—a sulfhydryl compound, ergothionine, a thiazole derivative, thiamin, sulfonic derivatives, and sulfate esters,—are known. Of these, allyl isothiocyanate, vinyl sulfide, and allyl sulfide are abundant in only a few plant species, whereas cystine, methionine, and sulfates occur generally in higher plants in appreciable amounts.

It can readily be shown that the deposition of selenium does not quantitatively follow that of sulfur and that analogous compounds are not present in the same ratios. In cereals Painter and Franke (145) found the mole S:Se ratios to vary little, as the following illustration will show:

	WHOLE GRAIN	CRUDE GLUTEN	GLIADIN	GLUTENIN
Mole S:Se ratio	159	165	174	145

With *Oönosis condensata*, an indicator plant, the mole S:Se ratio in the stems and leaves was 6.2, but in the roots it was 2.8. The same plant contained sulfate, but no selenate could be demonstrated (145). The presence of inorganic selenium in plants has not yet been proved, and only metallic selenium has been indicated. Except in the root systems of plants growing in selenized nutrient solutions or soil cultures, the selenium in plants seems to be present in organic forms.

II. METHODS OF ANALYSIS

Selenium could be easily determined where there was enough to weigh, but in plants, soils, and animal products which contained a few parts per million, new methods were essential. Since selenium in biochemical products appears to be in an organic form, oxidation is a necessary step.

TABLE 4
Comparison of methods for the determination of selenium

COMPOUND	SELENIUM CONTENT		
	Parr bomb method	Distillative method	Theory
	per cent	per cent	per cent
Diselenodiacetic acid	57.0	57.3	57.2
β, β' -Diselenodipropionic acid	52.2	51.9	52.0
Seleninoacetic acid	45.2	44.9	46.2
β -Seleninopropionic acid	42.4	41.1	42.7
<i>n</i> -Propylseleninic acid + HNO ₃	36.1	36.1	36.2
Benzyl selenide	30.1	30.5	30.3
Benzyl diselenide	46.4	46.5	46.4
β -Selenodipropionic acid . .	35.1	36.1	35.1

Horn (90) applied the codeine sulfate method to a sulfuric acid digest of plants as a qualitative test, but Martin (121) and Gortner and Lewis (85) report quantities from colorimetric comparisons. The reviewer has found that there is a loss of selenium by oxidation in sulfuric acid; hence the results obtained by this method are likely to be low when applied to materials which are difficult to digest to a clear solution.

The method of Robinson *et al.* (158) obviates the objection just stated, because the digestion is carried out in a closed system with the vapors, which contain some selenium dioxide, passing through a cooled bromine-hydrobromic acid solution. This method of digestion, which is similar to that of Fredga (76), has been widely used in the determination of selenium in plants and soils.

In order to compare the toxicity of selenium in plants with that of inorganic selenium salts, the method for determining selenium in plants must be accurate. Therefore the selenium content of several organic

compounds was determined (Painter, unpublished work) by the distillation method of Robinson *et al.* (158) and by the Parr bomb method, which Shaw and Reid (162) have found to be dependable.

From this comparison it can be assumed that a quantitative recovery of selenium is accomplished by the distillation method. The danger of the loss of volatile selenium bromide from the receiving flask would be greater when determining selenium in cereals, forages, or animal products, because the time required for digestion is much greater than with small samples of organic selenium compounds.

At the temperatures used in the modified methods of Dudley and Byers (48) and of Williams and Lakin (185), which are applicable to biological materials high in water, there is slight danger of loss of oxidized selenium. Dudley (45) has devised a method for the determination of selenium in air-gas-dust mixtures.

The colorimetric comparison of colloidal selenium is not entirely satisfactory, because the probable error in the analysis of plants is large. Franke, Burris, and Hutton (59) have improved the method for smaller quantities than can be compared accurately in solution. With quantities too large for satisfactory comparison of colloidal selenium, the volumetric method of Beath, Eppson, and Gilbert (9) is applicable. Application of the titration of metallic selenium by iodate or bromate and of selenite by thiosulfate, as outlined by Coleman and McCrosky (38), should increase the precision of the determination of selenium in plants.

III. ORGANIC COMPOUNDS OF SELENIUM

It is not in the scope of this review to list all of the known organic compounds of selenium or all methods which have been used in their synthesis. For these, reference is made to the reviews of Bradt and coworkers (20, 21, 22, 23, 24, 25). In the dissertations of Fredga (76) and van Dam (40), special phases of the chemistry of organic selenium compounds are discussed. A few general methods of preparation and a few reactions of each group of organic selenium compounds will be given.

The known types of organic selenium compounds are similar to those of sulfur. Organic selenols and selenides form insoluble complexes with mercury and with some other heavy-metal salts similar to the corresponding sulfur compounds. The general reactions of organic selenium compounds are similar to those of organic sulfur compounds, but selenium exhibits more metallic properties than sulfur. Selenite and selenate, or corresponding organic derivatives, are oxidizing agents, whereas reduced selenium is readily oxidized to the metallic form if inorganic and to diselenide if a selenol.

Metallic selenides or diselenides, selenocyanates, elementary selenium, selenium dioxide, selenium oxychloride, and selenium halides react with appropriate compounds to introduce selenium into organic molecules. Hydrogen selenide, which is necessary in many methods, is conveniently prepared by passing hydrogen through a hot suspension of selenium in a heavy motor oil (86).

A. SELENIDES

The following methods, which involve the direct introduction of selenium, are those usually used in the preparation of selenides:

- (1) $2RX + M_2Se \rightarrow RSeR + 2MX$ (76, 98, 128, 155, 167, 177)
- (2) $RSeM + R'X \rightarrow RSeR' + MX$ (15, 55, 79, 81, 105, 116, 150, 161)
- (3) $RMgX + Se \rightarrow RSeMgX$ (105, 151, 172)
 $2RSeMgX \rightarrow RSeR + Se(MgX)_2$
- (4) $RSeMgX + R'X \rightarrow RSeR' + MgX_2$ (33)
 $RMgBr + R'SeBr \rightarrow RSeR' + MgBr_2$ (13)
- (5) $2RNNX + M_2Se \rightarrow RSeR + N_2 + 2MX$ (113, 116)
 $RNNX + MSeR' \rightarrow RSeR' + N_2 + MX$ (104, 105, 106)
- (6) $RNNX + MSeCN \rightarrow RSeCN + N_2 + MX$ (16, 36, 79, 81, 101, 105, 106, 114)
 $PhNH_2 + Se_3(CN)_2 \rightarrow p-H_2NPhSeCN$ (36)
 $RSeCN + R'X + KOH \rightarrow RSeR' + KX + HOCN$ (101)

Aliphatic selenides are readily prepared by method 1, but in method 2 difficulties are encountered, because selenols are readily oxidized to diselenides on exposure to air and some selenols are unstable in alkaline solution. Methods 4 and 6 are limited to compounds of $R'X$ with active halogens, and method 3 is more often applied to the preparation of selenols than of selenides.

Selenoxides and many selenonium compounds give selenides on reduction. Diselenides also give selenides at high temperatures.

Selenides are the most stable class of organic selenium compounds. All but about sixty of more than two hundred known selenides (20) are aryl, heterocyclic, or mixed alkyl-aryl, alkyl-heterocyclic, and aryl-heterocyclic derivatives.

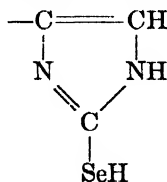
B. SELENOLS AND DISELENIDES

Since selenols and diselenides are reversibly interconvertible, methods for introducing selenium into organic molecules are often identical. The following methods include those generally used:

- (1) $RX + MSeH \rightarrow RSeH + MX$ (79, 123, 177)
 $2RSeH \xrightleftharpoons[2H]{\frac{1}{2}O_2} RSeSeR + H_2O$
- (2) $RSeMgX + HX \rightarrow RSeH + MgX_2$ (55, 105, 114, 172)
- (3) $2RX + M_2Se_2 \rightarrow RSeSeR + 2MX$ (40, 76)
- (4) $RX + MSeCN \rightarrow RSeCN + MX$ (16, 72, 76, 79, 114)
 $RSeCN + MOH$ (or HX) $\rightarrow RSeH + HOCN$
- (5) $2RNNX + M_2Se_2 \rightarrow RSeSeR + N_2 + M_2X$ (116)
- (6) $Se_2X_2 + 2RMgX \rightarrow RSeSeR + 2MX_2$ (169)
- (7) $K_2SeSO_3 + RX \rightarrow KSO_3SeR + KX$
 $2KSO_3SeR \rightarrow RSeSeR + K_2S_2O_6$ (79)

Methods 1, 2, 3, and 4 have found general application when R is aliphatic, and methods 2 and 5 when R is aryl. In method 4 the halogen must be active. There is good evidence that some diselenide of the type $R_2Se=Se$ is formed (76) with $RSeSeR$ in method 3.

Only eight of about thirty known selenols are aliphatic. Two selenols (22) which are imidazole derivatives of the type



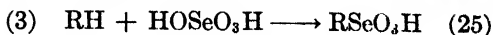
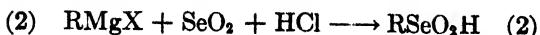
have been described. Mono-, di-, and tri-selenoglycerols have been prepared by Baroni (7), by the use of reactions similar to those in the preparation of the thio derivatives. Wrede (186) has prepared carbohydrates with a hydroxyl group replaced by SeH in the 6-position. Diselenides are more stable than selenols; hence more of them (about fifty) are known. Nearly all are homocyclic, aryl, and heterocyclic derivatives.

Fredga (76) and Backer and van Dam (2) have separated the *d*- and *l*-forms of optically active diselenides. They give surprisingly high values for optical rotation, like those obtained with disulfides.

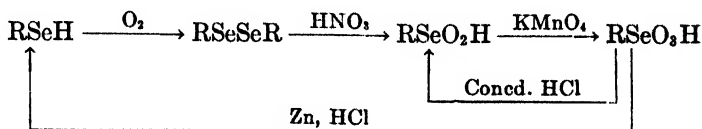
C. ORGANIC SELENIUM ACIDS

The more common members of this class, the seleninic and selenonic acids, are conveniently prepared by the following methods:

- (1) $\left. \begin{array}{l} RSeH \\ RSeSeR \\ RSeCN \end{array} \right\} \xrightarrow[HNO_3]{H_2O_2; KMnO_4; HNO_3} RSeOOH$
 $\xrightarrow{KMnO_4; Cl_2 \cdot H_2O; 30\% H_2O_2 \text{ in } CH_3COOH; K_2CrO_4} RSeO_3H$
 (3, 40, 76, 116, 161, 168, 154)



In the stepwise oxidation of selenols, each compound can be isolated, but the reduction is difficult to control.



Bradt and Valkenburgh (25) list twenty seleninic acids and thirteen selenonic acids. In all but seven, the selenium is attached to the benzenoid ring. Several seleninic acids, mostly derivatives of organic acids, were later described by van Dam (40) and by Fredga (76). Banks and Hamilton (6) have described amides of seleninic acids.

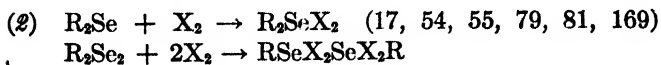
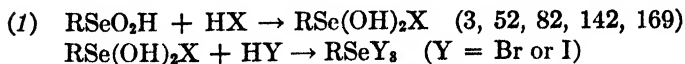
A notable difference in the ease of oxidation of sulfur and of selenium is evident from the methods used to prepare organic sulfur and selenium acids. Reagents which oxidize sulfhydryl groups and disulfides to sulfonic acids do not oxidize selenols and diselenides to selenonic acids, but only to seleninic acids. Seleninic acids are then much easier to prepare than sulfinic acids, but, like sulfinic acids, they are not stable. The acid salts of seleninic acids,—the selenonium compounds,—are more stable than the free acids. Selenonic acids, which require strong oxidizing reagents for their preparation, are more stable than seleninic acids, but both are strong oxidizing agents when compared with sulfur compounds of the same valence.

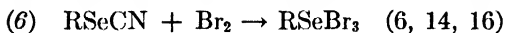
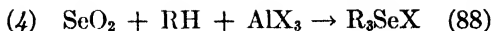
One selenol acid, $\text{C}_6\text{H}_5\text{C}(=\text{O})\text{SeH}$, the acid amides of five seleno acids, $\text{RC}(=\text{Se})\text{OH}$, and the potassium salt of the ethyl ester of selenol carbonic acid (25), potassium selenoxanthogenate, have been described.

D. SELENIUM COMPOUNDS

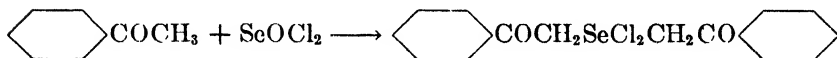
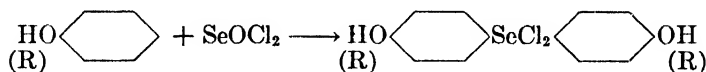
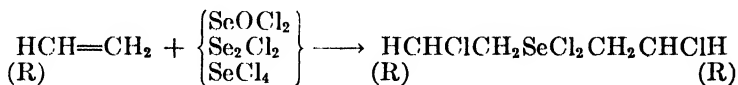
All compounds of the class RSeX_3 , R_2SeX_2 , R_3SeX , and $\text{RSeX}_2\text{SeX}_2\text{R}$ (R = an alkyl, aryl, or heterocyclic group; X = Cl, Br, I, OH, or NO_2) are classified as selenonium compounds. More than three hundred are known, so this is the largest group of organic selenium compounds.

Only a few of the many methods of preparation are listed:

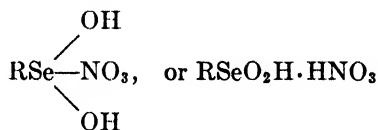




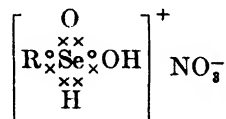
The reactions of SeOCl_2 , Se_2Cl_2 , and SeCl_4 with olefins (17), phenols (127, 140), phenol esters (1), and alkyl-aryl ketones (139) all yield selenonium compounds.



The formation of selenonium compounds from seleninic acids, particularly when excess nitric acid is used to oxidize selenols or diselenide, has been used as an explanation for the failure of nitric acid to oxidize seleninic acids to the selenonic acids (161). The structure usually given for these compounds

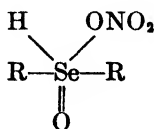


may, when the electronic structure of selenium is considered, be written



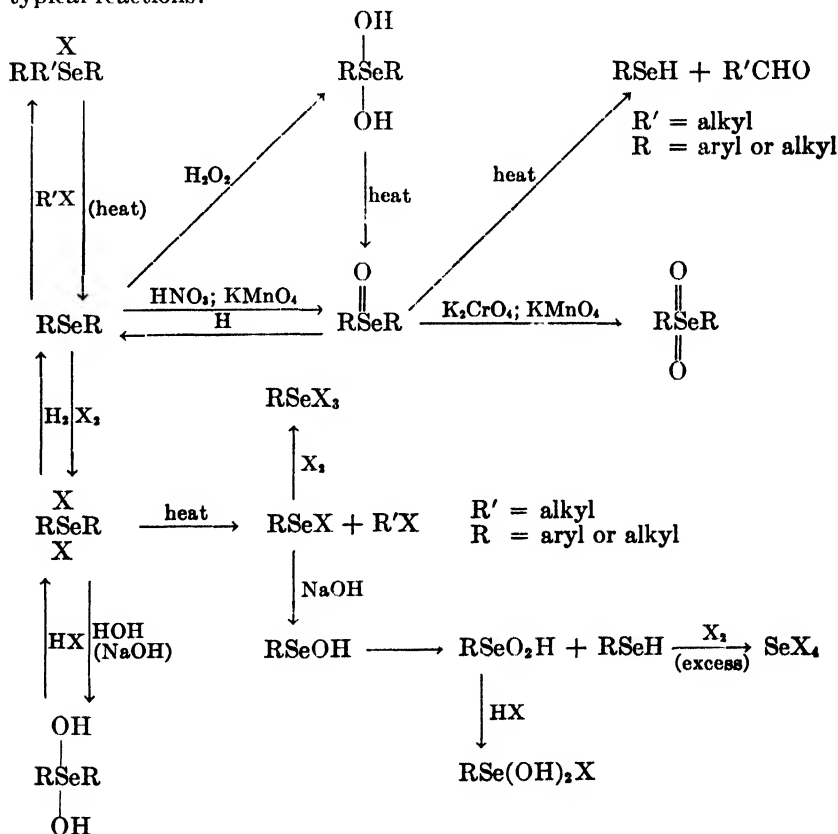
Selenonium compounds possess ionizable groups, and seleninic acids form salts with both acids and bases.

Similarly to its action on selenols or diselenides, nitric acid oxidizes selenides to selenoxides but not to selenones. Foster and Brown (55) suggest that a compound with hexavalent selenium



is formed, which prevents further oxidation. They state that on neutralization the selenoxide may be oxidized to the selenone, but few selenones (81) have been reported. Since these compounds and the dihalides of selenium ethers ionize in solution, it seems that they should be considered salts of a similar type, i.e., as acid addition products of seleninic acids. With halogen acids selenoxides give dihalides.

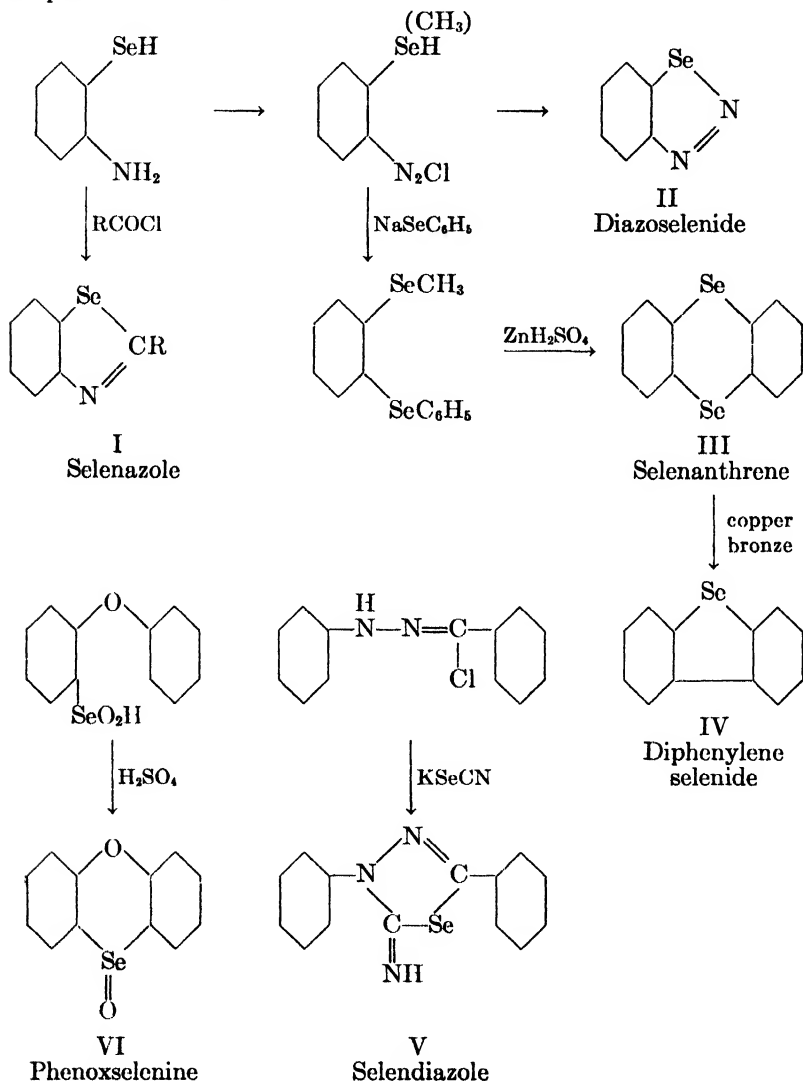
Although the stability of selenonium and related compounds is dependent upon the radical attached to selenium, the following may be considered typical reactions:



If one R is aliphatic, the compounds are less stable (13, 52); hence cleavage to a selenol or monohalide may occur. Selenols or diselenides give similar reactions, but when there is only one organic group attached to the selenium atom there is one more position at which a reagent may be added.

Sulfoxides of the type $\text{R}'\text{SR}$ can be resolved, but Gaythwaite, Kenyon,

and Phillips (81, 82) were unable to resolve selenoxides; this indicates that the linkage between selenium and oxygen may not be an unsymmetrical semipolar double bond.



E. COMPOUNDS WITH SELENIUM IN RING SYSTEMS

Many compounds with selenium in ring systems have been prepared. Bradt lists these with selenides, diselenides, or selenonium compounds, and most methods of preparation have been given under these classes.

Selenophene (26, 27, 171, 179) has properties similar to those of thiophene, for both are more stable to reagents than would be expected. A series of cyclic selenides,—cycloselenopropane, cycloselenobutane, cycloselenopentane, and cycloselenohexane and their derivatives,—were described by Morgan and Burstall (128). Substituted compounds of selenazole (I) (12, 37, 51, 108, 107), diazoselenide (II) (100), selenanthrene (III) (39, 100, 102, 103), diphenylene selenide (IV) (39), selendiazole (V) (80), phenoxselenine (VI) (173), selenoxanthene (21), selenoxanthone (56, 116, 173), selenonaphthene (110, 115), phenarsenazine, a seleno methylene blue (21), selenothiana (83), 1,4-selenoxane (83), a pelletierine derivative, 3-selen-9-azobicyclo-(3,3,1)-nonane (19), and a spiro compound, 2,6-diseleno-4-spiroheptane (4), are known.

A few diselenides in ring systems have been prepared by Fredga (73, 76) and by Backer and Winter (4).

F. MISCELLANEOUS ORGANIC SELENIUM COMPOUNDS

Many seleno aldehydes and ketones have been prepared (24), but it is doubtful if monomers have been studied, because of the tendency for these compounds to polymerize. The usual method is to add hydrogen selenide to an inert solvent containing an aldehyde or ketone and hydrogen chloride. Shaw and Reid (161) have described several selenomercaptols.

One selenonium selenol ($R_3\text{SeSeH}$) has been reported (24), but no selenide selenol (RSeSeH) is known.

Ethyl derivatives of $-\text{S}-\text{Se}-\text{S}-$ and of $-\text{Se}-\text{S}-\text{Se}-$ and a triselenide, $-\text{Se}-\text{Se}-\text{Se}-$, have been prepared by Levi and Baroni (117) by the reaction between ethyl selenol or ethyl mercaptan and thionyl chloride or selenium oxychloride.

Several selenium compounds which also contain mercury, arsenic, or antimony are of interest because of their possible use in therapeutics (109), and several benzanthrone derivatives (50, 174) have valuable properties as dyes.

IV. THE PROPERTIES OF SELENIUM IN PLANTS AND THEIR RELATION TO KNOWN COMPOUNDS OF SELENIUM AND OF SULFUR

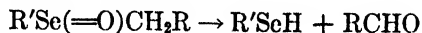
From the properties of selenium in naturally occurring plants it is generally agreed that the selenium is in organic forms. Franke and Painter (63) were unable to extract the selenium from cereals by solvents for inorganic selenium salts or for metallic selenium. Electrodialysis of peptized seleniferous proteins likewise failed to remove selenium. Much of the selenium in the indicator plants is water-soluble but cannot be reduced to metallic selenium by reagents which reduce inorganic selenite or selenate. Not one of the organic selenium compounds studied by Painter, Franke, and Gortner (147) yielded more than a trace of metallic selenium on reduc-

tion. They were reduced instead to selenides, diselenides, or selenols, which are more stable forms than are the oxidized organic selenium compounds.

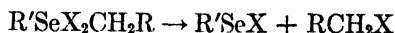
The only form of inorganic selenium reported in plants is metallic selenium. Several investigators (for references see Hurd-Karrer (97)) grew plants in artificially selenized soils or cultures, using selenite, and reported that metallic selenium was deposited in the growing plant, particularly in the root systems. Levine (118) states that reduction is probably due to microorganisms, but it is well known that many compounds in plants,—i.e., glutathione, ascorbic acid, reducing sugars, etc.,—can reduce selenite to elemental selenium. In view of these facts it may seem surprising that metallic selenium has not been found in naturally occurring seleniferous plants, but we have no proof that selenium is absorbed from naturally seleniferous soils as selenite nor is the evidence for the presence of elemental selenium conclusive. Plants grown in selenite cultures may have an abnormal reddish cast and the selenium may be extracted from the roots by a bromine-hydrobromic acid solution, as Hurd-Karrer (97) found, but that is not proof. Metallic selenium and inorganic selenite and selenate are readily converted by bromine-hydrobromic acid solutions to selenium bromide, which can be distilled, but this reagent also converts many organic selenium compounds to soluble compounds which can be cleaved to inorganic forms of selenium. This property has apparently been overlooked. The results of Westfall and Smith (180), who, after extraction of naturally seleniferous cereals and proteins by dilute bromine in hydrobromic acid or by hydrogen peroxide in trichloroacetic acid and distillation of the extracts in the presence of bromine-hydrobromic acid, were able to reduce selenium in the distillates to the metallic form, can be explained by oxidation and cleavage to inorganic forms and need not be interpreted to indicate the presence of inorganic selenite or selenate, as suggested. Painter (142) found the selenium in *Oenopsis condensata* to be cleaved by bromine-hydrobromic acid solutions to a form reducible by sulfur dioxide and hydroxylamine hydrochloride. The reduction of selenium extracted from indicator plants to the metallic form has not been found to occur without previous oxidation and cleavage.

These results are explained by the reactions of organic selenium compounds. Selenides add bromine to form dibromides, which give dihydroxides in the presence of excess water. Peroxides also convert selenides to dihydroxides. Dihydroxy derivatives of selenium ethers are converted to selenoxides on heating. These selenonium compounds of the type $R'SeX_2R$ [$X_2 = Br_2, Cl_2, I_2, (OH)_2, \text{ or } O$] are easily decomposed if R is aliphatic, as Edwards, Gaythwaite, Kenyon, and Phillips (52) and Be-

haghel and Hofmann (13) have shown. Selenoxides, like sulfoxides, cleave mainly as indicated by the equation



Dihalides and dihydroxides cleave in a similar manner:



With dihydroxides the diselenide (52) was isolated. With some compounds high temperatures were required, but with others the dihalides or dihydroxides decomposed so readily that they have not been isolated. The author (unpublished work) dissolved selenides in bromine-hydrobromic acid solution, distilled until the solution was free of bromine, and recovered inorganic selenium in the distillates. In the presence of excess bromine or peroxide in aqueous solution the cleavage products of each reaction,—RSeH, R'SeX, and R'SeSeR',—would give a seleninic acid. Excess bromine converts each to a tribromide, RSeBr₃, which in aqueous solutions goes to a seleninic acid. Peroxides convert diselenides directly to seleninic acids. Painter (142) and Painter, Franke, and Gortner (147) have shown that seleninic acids cleave to give mostly inorganic selenite. Indeed, the results of Westfall and Smith, when considered with other properties of selenium in cereals, can be better interpreted to indicate the presence of a diselenide or of an easily cleaved selenide.

If elemental selenium sometimes occurs in plants—and this appears likely—it behaves differently from sulfur. No report of elemental sulfur in higher plants is known to the author. We must recognize that when elemental selenium was reported to be deposited, it was from absorbed selenite, which is easily reduced, whereas sulfur is absorbed by plants as sulfate.

Although Painter and Franke (143, 145) found most of the selenium in wheat, corn, and barley to be confined to the protein, Beath, Eppson, and Gilbert (10) found about half of the selenium in a wheat sample to be water-soluble. Whether or not the selenium not accounted for in the proteins (145) isolated,—approximately 20 per cent of the total,—was in a non-peptized or a water-soluble protein which is richer in selenium than the proteins isolated, cannot be stated. The mole N:Se ratio in most of the proteins (145) was higher than the N:Se ratio of the whole grain. This suggests that there is some non-protein selenium present. As previously stated (145), the S:Se ratios in the grain and proteins were fairly constant, indicating that selenium deposition follows that of sulfur more closely than that of nitrogen. The water-soluble selenium in the indicator plants (8, 10) is probably not in proteins, although many of these plants

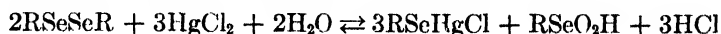
(legumes) contain a large amount of water-soluble protein. In any case there is no good evidence to indicate that the selenium is inorganic.

The fact that the selenium in cereals is in the protein is in itself presumptive evidence of the presence of a nitrogen compound of selenium, probably an amino acid. After the hydrolysis of seleniferous proteins, Painter and Franke (143, 144, 146) and Horn, Nelson, and Jones (92) found, in the hydrolysate, soluble selenium compounds which could not be reduced to elemental form. The humin formed when seleniferous proteins were hydrolyzed (144) by 20 per cent hydrochloric acid or by 33 per cent sulfuric acid always contained selenium. Recently, Schaefer and Moxon (personal communication) boiled the selenium analog of cystine in the same concentration of acids that Painter and Franke used to hydrolyze proteins and noted a slow decomposition accompanied by the separation of selenium from solution. Selenium is less stable than sulfur to acid hydrolysis, because the mole S:Se ratio in the humin (144, 145) is lower than in the protein or hydrolysate. Westfall and Smith (180) cleaved more selenium than sulfur from seleniferous proteins by oxidizing them in acid solutions. The amount of selenium in the humin, as well as of sulfur, could be increased by the addition of carbohydrate or by the use of stronger acids. The observation of Painter and Franke (144), that the hydrolysis of seleniferous proteins in concentrated hydriodic acid and removal of the hydriodic acid by repeated extraction with ether gave a selenium-free hydrolysate, is of interest. Hydriodic acid cleaves ethers and when used to hydrolyze proteins cleaves the methiol group of methionine to form an α -amino γ -thio lactone.

Several attempts have been made to separate selenium compounds from protein hydrolysates. Horn, Nelson, and Jones (92) were able to extract the selenium by means of butyl alcohol. The monoamino monocarboxylic acids are generally considered to be extracted by butyl alcohol, but the amino acids extracted depend largely on the pH of the solution. Only a little selenium was present in the dicarboxylic acid (92) fraction. The hexone bases contained small amounts of selenium (92), but Painter and Franke (143) found considerable selenium in the phosphotungstic acid precipitate of a protein hydrolysate. Mercuric chloride (143) was the most effective of several amino acid precipitants used to remove selenium from protein hydrolysates. Copper salts precipitated some selenium compounds but silver salts, which precipitate histidine and arginine, removed only a trace of selenium. As a result of these studies, it can be concluded that the selenium compounds do not show properties identical with those of any known amino acid. It is of interest to note that every amino acid fraction which contained cystine or methionine also contained selenium, although not in the same S:Se ratio. When Jones, Horn, and Gersdorff

(99) separated partially hydrolyzed products of the enzymatic hydrolysis of seleniferous protein, they found selenium to be in those products which contained cystine.

When the selenium-containing precipitates of mercury and copper salts from a protein hydrolysate were decomposed with hydrogen sulfide, much selenium was in the metallic sulfide. Fredga (74, 75, 76) and Preisler (153) studied the action of metallic salts on diselenides of organic acids. The compounds dismute according to the general scheme:



A seleno mercaptan which is not decomposed by hydrogen sulfide may form when heavy-metal salts are added to a seleniferous protein hydrolysate, or a seleninic acid may form which is cleaved and the selenite may be reduced by hydrogen sulfide.

Like sulfur in proteins, some selenium is cleaved when seleniferous proteins are hydrolyzed in alkaline plumbite. The percentage of total selenium in the lead sulfide was always slightly less than the percentage of total sulfur in the lead sulfide, thereby indicating that selenium is more stable in alkaline solutions than is sulfur. The following comparison from tables by Painter and Franke (146) is typical of several proteins:

PROTEIN	SELENIUM	SULFUR	LEAD SULFIDE PRECIPITATE		FILTRATE FROM LEAD SULFIDE	
			Se	S	Se	S
	<i>p.p.m.</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Gluten . .	117	0.74	33.3	52.8	65.0	46.4

Methods of alkaline hydrolysis which produced a higher percentage of lead sulfide also removed a greater percentage of selenium in the lead sulfide precipitate. The cleavage of diselenides in alkaline solutions (147) is similar to that of disulfides. Inorganic selenide, which precipitates as lead selenide in the presence of plumbite, and some selenite form. There is good evidence that some inorganic selenide forms in the absence of lead when seleniferous proteins are hydrolyzed in alkaline solutions (146), but inorganic selenite has not been demonstrated. This may be because the methods are inadequate for detection of the different forms of cleaved inorganic selenium in the dilute solutions. Most of the proteins contained from 100 to 150 p.p.m. of selenium.

The selenium in some of the high absorbers of selenium exhibits a different behavior in alkaline solutions from the selenium in the cereal proteins. Painter (142) dissolved nearly all of the selenium by aqueous sodium hydroxide extraction of *Oenopsis condensata*, but only a trace of

"labile selenium" separated when heated in alkaline plumbite. No selenium was cleaved to selenite. The plant contained 1180 p.p.m. of selenium, so if selenium underwent cleavage, there should have been little difficulty in detecting the different inorganic forms. The organic sulfur content of the plant was 0.17 per cent, but there was only a trace of "labile sulfur."

Additional indication of the probable types of selenium compounds in plants may be gained from the studies of Painter, Franke, and Gortner (147) on organic selenium compounds. Most of the selenium was cleaved in alkaline solutions from diselenodiacetic acid and β, β' -diselenodipropionic acid. Benzyl and *n*-propyl diselenides were partially cleaved. Selenium ethers were stable, with the exception of β -selenodipropionic acid which gave lead selenide almost quantitatively in alkaline plumbite. Seleninic acids, except *n*-propylseleninic acid, which was stable, gave mostly inorganic selenite. Since most seleninic acids, especially derivatives of organic acids, are unstable and are cleaved in acidic as well as in basic solutions, it is improbable that they occur in plants.

From the properties of selenium in cereals it would appear that the selenium is present in stable compounds. If the selenium is an integral part of the protein molecule, it would be expected to undergo few changes without hydrolyzing the protein. There is a general belief among farmers that seleniferous plants become less toxic upon long storage. Franke and Painter (66) found that a wheat sample decreased in toxicity after several years of storage. Moxon and Rhian (135) repeated the determination of selenium in some cereals after an interval of two to three years and found a consistent decrease of approximately 30 per cent in the selenium content. If selenium were slowly oxidized, there would probably be cleavage, but organic selenium compounds are not oxidized as readily as organic sulfur compounds. If a dismutation of diselenides took place, slow loss of selenium would occur, but this seems doubtful in cereal grains under dry storage conditions. The organic selenium compounds prepared by Painter (142) differ greatly in their stability, but few are as stable as selenium compounds in plants. Diselenodiacetic acid was much less stable than other diselenides. It deposited metallic selenium rapidly when exposed to air. Seleninic acids decomposed more readily than the acid salts,—the selenonium compounds. No decomposition of selenium ethers has been noted after more than three years' storage.

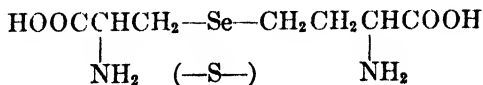
In the group of indicator plants which accumulate large amounts of selenium, volatile compounds of selenium are present. A large percentage of selenium (10) may be lost on drying these plants when they are collected green. These volatile selenium compounds may impart a disagreeable odor to some plants, and seleniferous *Astragali* may be detected from the

odor. Volatile sulfur compounds abound in some plants, but most of the indicator plants belong to a different family from those which are considered the best sources of volatile sulfur oils.

It may be that the slow loss of selenium from cereals is due to volatile compounds, but nothing abnormal about the odor or taste of seleniferous cereal grains has been observed. In this connection the ability of rats, and presumably of farm animals as well, to distinguish between foods of different selenium content and foods free from selenium (69) is of interest. In the experiments of Franke and Potter (69), wheat and selenite were the sources of selenium. A pungent selenide odor similar to, if not identical with, that exhaled by animals which have been injected with inorganic selenium salts, was given by a calcium hydroxide hydrolysate of a seleniferous protein (142) when treated with a current of carbon dioxide.

When the properties of selenium in the few plants which have been studied are considered together and are compared with the general properties of organic selenium compounds, the evidence, although fragmentary, points to definite types of compounds. There is good evidence for the presence of selenides and diselenides, but not for seleninic or selenonic acids. Aside from the fact that most of the oxidized selenium compounds undergo cleavage, it seems likely that they would be reduced by metabolic processes in the plant. Different organic compounds must occur in some of the plants which accumulate larger quantities of selenium than commonly occur in cereals. The types indicated are analogous to those of the naturally occurring sulfur compounds. The failure to find selenate in plants is not surprising when it is considered from a chemical standpoint. When selenate is absorbed by plants, a preferential reduction of selenate over sulfate should occur. It is likely that some selenium would be reduced to the elemental form without forming carbon selenium bonds, especially when high concentrations of selenite are absorbed.

A crystalline selenium-containing amino acid has been recently isolated by Horn and Jones (91), and it is of additional interest that their preparation also contains sulfur. The structure suggested by the empirical formula,



differs from that of any known sulfur-containing amino acid. The authors do not state the source of their amino acid.

Analogous selenium and sulfur compounds possess similar properties, so if they occur in plants, the difficulty of separating these in the cereals where the molar S:Se ratio is rarely less than 100 is obvious. In the

indicator plants where as high as 15,000 p.p.m. of selenium has been reported, the S:Se ratio must be less than 1 in some samples. The advantage of working with these plants is obvious, but, since little is known about the chemistry of these plants, wholly different compounds from those in cereals may be present. Studies of the properties of the selenium analogs of the sulfur-containing amino acids should answer a fundamental question regarding selenium in cereal grains. Selenocystine is the only selenium analog of the naturally occurring organic sulfur compounds which has been prepared. Fredga (77) studied the reaction between the methyl ester of α -amino- β -chloropropionic acid (from serine ester) and potassium selenide, and Painter (142) treated the chloro ester with sodium hydroselenide and oxidized the selenol to the diselenide. After hydrolysis, selenocystine precipitated when the solution was neutralized. As expected, selenocystine gave "labile selenium" in alkaline solution.

In addition to supplementing the chemical evidence that the selenium in plants is organic, animal experimentations also indicate that selenium- and sulfur-containing amino acids are related in metabolism. The toxicity of *d,l*-selenocystine (table 1) was much greater than that of any other organic selenium compound when injected. It was nearly as toxic as inorganic selenite. Elemental selenium, reported to be in plants, was virtually non-toxic when fed to rats (68), and the selenium in cereals was more toxic than any inorganic form of selenium studied. Both the *d*- and the *l*-forms of the selenium analog of cystine (separated by Fredga (78)) have been fed to rats. Moxon (129, and personal communication) found the toxicity of selenocystine to be in the same range as the selenium in cereal grains and the *l*-form more toxic than the *d*-form. This seems significant when it is recalled that the naturally occurring amino acids are of the *l*-configuration and that *l*-cystine is more efficient in supporting growth than is its optical isomer.

Further similarity in the behavior of selenium and sulfur in the animal body has recently been discovered by Moxon *et al.* (136). Bromobenzene lowered the selenium content in the blood and greatly increased its urinary excretion. Bromobenzene is known to become conjugated with tissue cystine and methionine and to be excreted in the urine in this form.

I wish to extend my appreciation to Professors R. A. Gortner and L. I. Smith for their interest and review of this manuscript, to Dr. C. F. Rogers for many valuable suggestions, and to A. L. Moxon for his suggestions on the sections on the toxicity and geology of selenium and for permission to quote work in advance of publication. Acknowledgement is also gratefully made to Ruth Robbins-Painter for aid in preparing the manuscript.

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A REVIEW OF SOME RECENT X-RAY WORK ON PROTEIN CRYSTALS

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Received September 14, 1940

This is a difficult moment at which to try to give an account of protein structure from the x-ray side. A great deal of work is in progress, and it seems likely that many points about which we now conjecture will be settled soon if only the work can go on.

Three crystalline proteins have been studied in some detail by crystallographic methods,—insulin, horse methemoglobin, and lactoglobulin,—and this report will be mainly concerned with their investigation. The measurements on hemoglobin¹ have been carried out by Dr. M. F. Perutz, working at Cambridge University under Professor W. L. Bragg with a grant from the Rockefeller foundation. As Dr. Perutz is now interned² and may be unable to send an account of his work himself, Professor Bragg has kindly given me permission to use his results. The work on insulin and lactoglobulin has been carried out at Oxford University.

These three proteins have the advantage that they have all been x-ray photographed in both the wet and the dry states, and lactoglobulin in particular in several crystalline modifications (table 1). Further, a number of measurements have been made of the intensities of x-ray reflections from these crystal structures, and several Patterson Fourier projections have been calculated for each protein (figures 1 to 7). Unfortunately only in the case of air-dried insulin are these calculations anything like complete.

The first question that it seems relevant to ask about these crystal structures is whether or not there actually exist in the crystal separate individual molecules of the magnitude deduced by Svedberg and his co-workers from ultracentrifuge measurements. In x-ray analysis no absolute proof of the presence of a molecule is possible without a complete analysis which will demonstrate the degree of chemical binding in different directions in the crystal structure from the lengths of the interatomic

¹ The Patterson projections for wet hemoglobin were calculated by Mr. D. P. Riley from data supplied by Dr. Perutz.

² Since released (note added February, 1941).

TABLE 1
X-ray measurements on the proteins insulin, hemoglobin, and lactoglobulin

PROTEIN	a	b	c	β	CELL VOLUME	DENSITY	SPACE GROUP	MOLECULES PER CELL	DIRE- FRIN- GENCE
Insulin (hexagonal axes)	83.0		34.0		203,000	1.28	R3	3	+
	74.8		30.9		150,000	1.31	R3	3	+
Insulin (rhombohedral axes)	49.4			$\alpha = 114^{\circ}16'$	68,000			1	
	44.4			$\alpha = 114^{\circ}28'$	50,000			1	
Horse methemoglobin	110	63.8	54.2	112°	352,000	1.242	C2	2	(?)
	102	51	47	130°	188,000	1.270	C2	2	
Lactoglobulin, tabular, orthorhombic	67.5	67.5	154	90°	702,000	1.257	P2 ₁ 2 ₁ 2 ₁	8	-
	67.5	67.5	148.5	90°	677,000		P2 ₁ 2 ₁ 2 ₁	8	-
	60	63	110	90°	416,000	1.27	P2 ₁ 2 ₁ 2 ₁	8	
	67.5	67.5	133.5	90°	608,000		P4 ₂ 2 ₁	8	+
Lactoglobulin, needle, tetragonal.	56	56	(130)	90°	408,000	(1.3)		8	

distances found. But the comparison of Patterson projections for wet and air-dried insulin (3) do provide strong evidence in favor of the Svedberg molecule in the crystal. In each of these projections there is a group of eighteen peaks around the origin, having the same positions relative to one another in both projections. The difference between the two projections is mainly due to a difference in the orientation of the adjacent groups of peaks. Figure 1 shows the effect. The whole group at the origin A is turned relative to the group at B through an angle of about 6° in the wet as compared with the dry structure. The group of eighteen peaks is there-

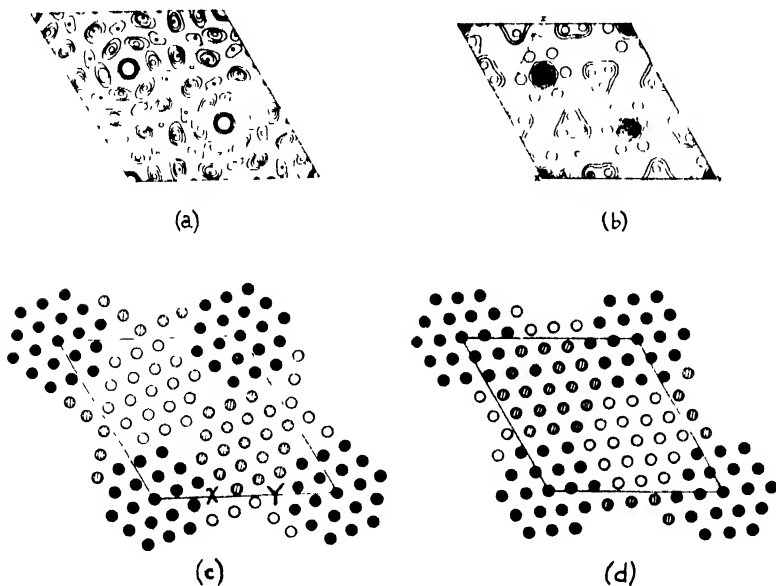


FIG. 1. Patterson projections on the basal plane of the structure of (a) wet and (b) dry insulin. Arrangement of hexagonal arrays of points: (c) expanded (*cf.* wet insulin) and (d) close-packed (*cf.* dry insulin).

fore interdependent, and the peaks move as if they corresponded to interatomic distances organized together in a single unit. The simplest interpretation of this organized unit makes it of the same size as the crystal unit cell, which in turn is equal in weight to one Svedberg molecule. One can derive somewhat similar evidence, though not so coherent, from a comparison of two of the lactoglobulin crystal structures (4). Here the crystallography is much more complicated. In the wet tabular crystals the molecular weight of the unit cell indicates the presence of eight Svedberg units plus a large proportion of liquid of crystallization. The crystals

shrink primarily in the direction of the *c*-axis, first by only 3.6 per cent to a partly wet form, and then by 11 per cent to the air-dried disordered structure. Comparison Patterson projections of the wet and partly wet structures show patterns which differ considerably more in the peak formations corresponding to large interatomic distances than in the arrangement of peaks about the origin. Such a distinction is in accordance

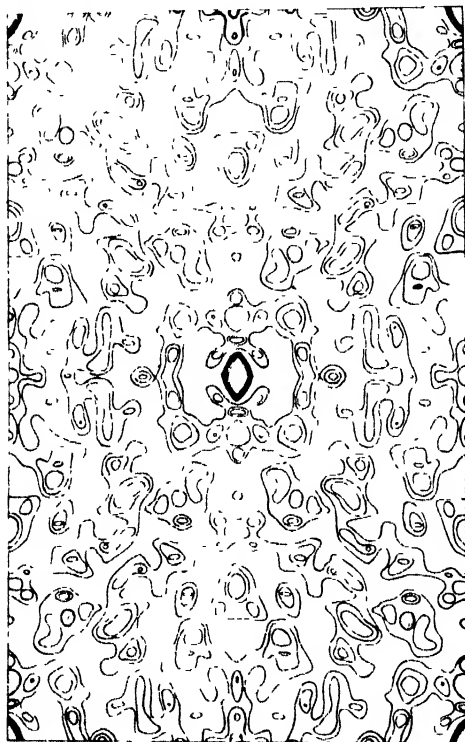


FIG. 2. P_{11} derived from wet crystals of horse methemoglobin (after M. Perutz and D. P. Riley)

with the presence of molecules which move closer together during the shrinking process, maintaining their internal structure relatively unchanged.

If we take the existence of protein molecules in the crystal as to some extent established, the next problem we have to consider is that of their shape. This question has recently received considerable attention from a number of workers. The most interesting calculations are probably those of Polson and Neurath, who have calculated the dissymmetry of



FIG 3

FIG. 3 Horse methemoglobin: Patterson projection P_{xz} from wet crystals (after M. Perutz and D. P. Riley).

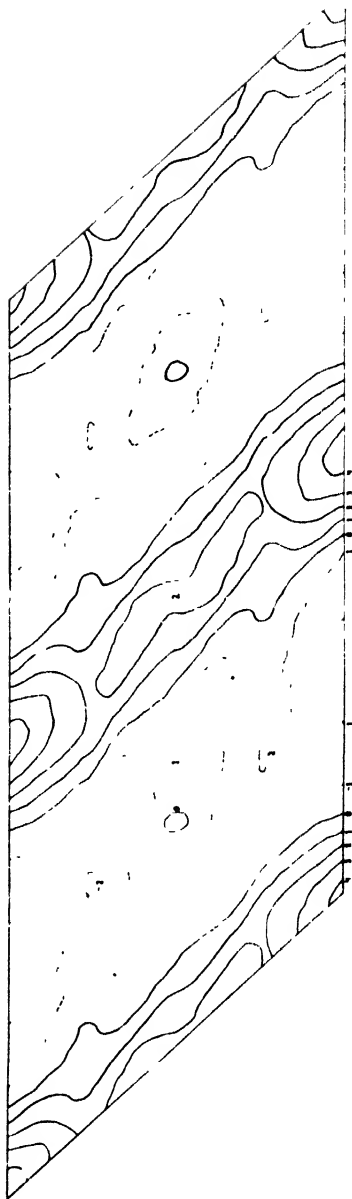
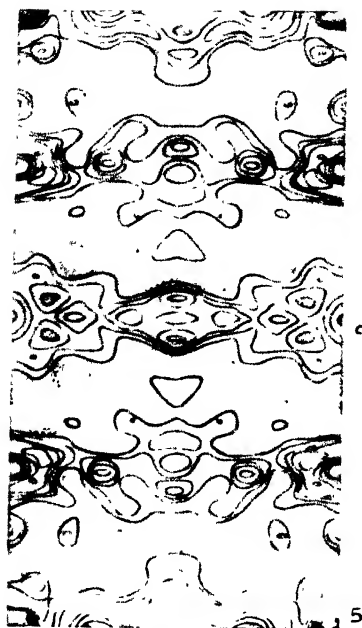


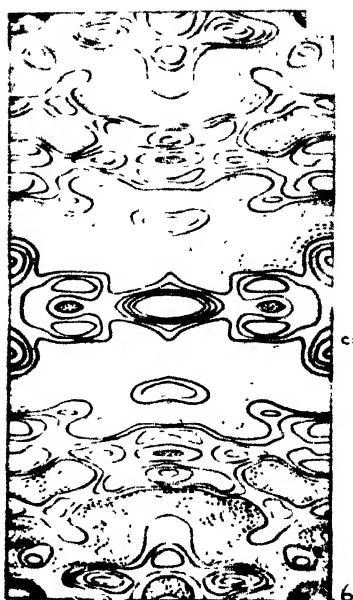
FIG 4

FIG. 4. Horse methemoglobin: P_{xz} derived from air-dried crystals, contour lines at 25 units apart (after M. Perutz)



$c = 154 \text{ \AA}$

5



$c = 167.5 \text{ \AA}$

6

$b = 67.5 \text{ \AA}$



7

FIG. 5. Lactoglobulin, tabular, wet; Patterson projection on (100)

FIG. 6. Lactoglobulin, tabular, partly wet; Patterson projection on (100)

FIG. 7. Lactoglobulin, tabular, wet; Patterson projection on (110)

protein molecules from the Svedberg dissymmetry constant, f/f_0 , obtained from diffusion constant measurements. Neurath (5), in particular, uses the dissymmetry constant to deduce possible dimensions of the protein molecules concerned, assuming these to be prolate ellipsoids of revolution. His values for insulin, lactoglobulin, and hemoglobin are as shown in table 2.

In these deductions it is assumed that the other factors which may influence the dissymmetry constant, the charge on the protein molecule and hydration, are negligible compared with the dissymmetry factor. Perutz has recently examined the other extreme hypothesis (which was first considered by Adair and Adair (1)),—namely, that the Svedberg dissymmetry constant is mainly a measure of the hydration of the protein mole-

TABLE 2
Dimensions of protein molecules

PROTEIN	f/f_0	b/a	a	b
Insulin	1.13	3.3	31	102
Lactoglobulin	1.2	4.3	28	122
Hemoglobin (horse).....	1.24	4.8	32	155

TABLE 3
Radii of proteins

PROTEIN	r_1 (DRY "RADIUS")	r_2 (WET "RADIUS")	r_3 (WET "RADIUS", CALCULATED FROM VOLUME OF UNIT CELL)
Insulin	22.4	25.8	25.3
Lactoglobulin	23.2	29.2	28.8
Hemoglobin	27.2	29.9 33.7 34.6	34.8

cule in solution. To conform to the observations, the protein molecules must increase in effective size and have a lower density on hydration. Perutz (6) therefore calculates first the radii of the dry proteins from their measured molecular weights and partial molar volumes, assuming they are spherical, and then the radii of the wet proteins required to agree with the diffusion constant measurements (see table 3). He shows that a considerable degree of asymmetry,—as much as 1.4 or 0.71,—may be present without appreciably affecting the dissymmetry constant, and that therefore these radii are not to be taken as an exact measure of the shape of the molecules concerned. The third column of table 3 gives r_3 , the radius of a hypothetical molecule, having the volume of the crystallographic unit cell found for the wet crystals, divided by the number of

molecules present. It is clear that the calculations show that the deviation of the dissymmetry constant from unity *could* be explained by hydration of the protein molecules in solution roughly equivalent to their hydration in the crystals.³

As both authors agree, in reality it is probable that both hydration and shape play a part in determining the observed dissymmetry constant. It should be possible to judge their relative importance by an examination of the x-ray evidence in more detail.

To take the case of insulin first. The unit cell of the air-dried crystals is a flat rhombohedron of very marked asymmetry. As this cell is of

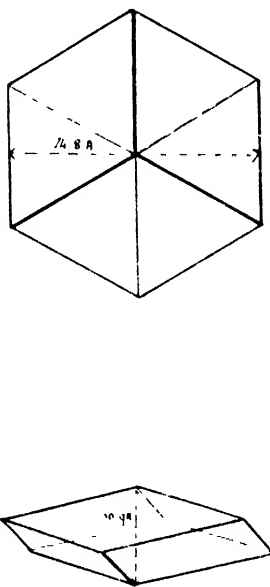


FIG. 8. The unit cell of air-dried insulin

weight equivalent to one Svedberg molecule, it might itself represent the shape of the molecule. But it is not possible actually to accommodate in it a molecule of the shape calculated by Neurath. If considered as approaching an ellipsoid of revolution, this ellipsoid would in the first case have to be oblate, not prolate, and its dimensions might be given roughly, to compare with Neurath's calculations, as follows: height, 30 Å.; diameter, 74.8 Å. (see figure 8). Actually, it is probable from the crys-

³ In this connection it is interesting that the direct calculation of the molecular weight of pepsin from diffusion constant measurements gives a value twice as large as that given in the ultracentrifuge, while the crystals contain roughly 50 per cent of liquid of crystallization.

tallographic placing of adjacent molecules that the molecule would not have so extreme a form. The most compact alternative is represented by a prism of hexagonal base 43 Å. across and height 30 Å., and the truth probably lies between these limits. The study of the Patterson projection (see below) suggests that the close packing of the molecules in the crystal is determined not simply by their approximate shape, but to a large degree by their internal structure.

The x-ray results therefore support Neurath's deduction,—namely, that the insulin molecule is markedly asymmetric,—while excluding such a high degree of asymmetry as that proposed. The additional deviation of the dissymmetry constant is probably due to hydration, as Perutz suggests. The comparison of the Patterson projections from wet and from dry insulin indicates that the molecules have moved from loose packing in the wet crystals to close packing in the dry crystals. The patterns suggest that in the wet crystals there are gaps at *X*, *Y*, etc. (figure 1) between the molecules which are filled with liquid of crystallization. It would be unlikely that this particular liquid would be carried with the molecules in solution. But the shrinking of the crystals on drying is greater than corresponds theoretically to the calculated shift in position of the molecules. The excess shrinking is due to hydration that might persist in solution and consequently further affect the diffusion constant.

The evidence from lactoglobulin and hemoglobin is less precise than that from insulin, owing to crystallographic complications, but it tends in the same direction. The Patterson projections from wet tabular lactoglobulin indicate that the molecules are packed in a pseudo-face-centered cubic array. Such an arrangement suggests that the molecules are not far from spherical, though a dissymmetry of the axes as great as 2:1 is possible,—again much smaller than that calculated by Neurath. It is also improbable that the hydration of the molecules in solution is quite as great as that of the wet tabular crystals, since these very easily shrink to the partly wet form, closely related in structure, and, further, the metastable needle crystals also have a lower hydration.

The crystal structure of hemoglobin presents some interesting problems. If the chemical molecular weight of 66,700 is accepted, the positions of the molecules are fixed in the lattice on twofold axes at the corners of the unit cell and *C* face centers. Perutz calculates that the most probable form of the molecule is a triaxial ellipsoid with $x = 22$, $y = 24$, and $z = 37.6$ Å. Setting $x = y = 23$ Å., the axial ratio is 1.63,—very much smaller than that found by Neurath. In fact, molecules of Neurath's calculated dimensions are, as in the case of insulin, excluded by the dimensions of the unit cell. As before, the hydration of the crystals is high, and probably a considerable proportion persists in solution.

Perutz proceeds to consider the methods of distributing the water of hydration. It is clear, as mentioned above, that this water must both increase the size and reduce the density of the molecules,—i.e., it does not simply enter into a central cavity of the molecule, as might be possible with molecules of the cyclol structure put forward by Dr. Wrinch. It may either be distributed over the surface of the molecule, or be disposed between structural units within the molecule in such a way that it causes an internal expansion. Or, of course, both these processes may occur. Perutz is rather in favor of the second hypothesis, to explain the fact that the introduction of heavy atoms into hemoglobin crystals through the liquid of crystallization produces no noticeable alteration in the intensities of x-ray reflections from the crystal. For insulin it should be possible to distinguish between the two alternatives through the calculated wet and dry Patterson projections on (0001). In the first case one would expect the characteristic peak system of dry insulin to be distorted in the wet crystal; in the second, the distances of the peaks from the origin should increase. Actually, for certain theoretical structures the two effects may lead to the same results and both changes appear to be present. Also, it is doubtful whether the accuracy of the present wet projection is sufficient for it to be employed in this argument, and better data are in preparation. One point is worth making,—the smallness of the change involved in atomic terms. The calculated change, if it is intramolecular in the projected distance of the inner insulin peak, is only from 10 to 10.5 Å. while, if the water of hydration is disposed over the surface of the protein molecule, it would correspond in quantity to a layer of water only one molecule thick.

The problem of hydration has an important bearing on that of the internal structure of the protein molecule, which is the third (and much the most speculative) point that I wish to discuss. The evidence to be considered here is mainly that provided by the Patterson projections of the different proteins.

These projections should first be distinguished according to resolving power. The limits of spacing used in their construction are roughly as follows:

	Å.
Wet hemoglobin	2.4
Wet insulin	4.5
Wet tabular lactoglobulin (100)	5.5
Partly wet tabular lactoglobulin (100)	6.5
Dry insulin	7.5
Wet tabular lactoglobulin (110)	10.5
Dry hemoglobin	13

As would be expected, the patterns for lactoglobulin (110) and for dry hemoglobin show broad, largely unresolved groups of maxima which prob-

ably indicate the main positions of the molecules, but little more. That from wet hemoglobin at the other end of the scale is the only projection which shows peaks as close as 4.5 Å. to the origin. The peaks are of very great interest, but it is impossible to be sure that the actual interatomic vectors concerned are 4.5 Å. until the third projection for this structure has been calculated. Apart from these features it is the similarities between the different projections that are most notable. Those of wet hemoglobin and insulin in particular show a general distribution of low maxima roughly in a series of rings about the origin with, very roughly, intervals of 10, 20, 30 Å., etc. In the lactoglobulin (100) projection the peak systems are only clearly resolved near the origin, where they show marked resemblances to the others.

A striking feature of the (0001) projection of air-dried insulin was pointed out by J. D. Bernal (2). In position all the observed peaks fall on a hexagonal network, the axes of which lie at an angle to the crystallographic axes. The angle observed is closely that required if the insulin molecule itself has a structure in which the eighteen points of the network around the origin are occupied by units which are arranged in a close-packed array, not only within one molecule but also with reference to the unit structure of neighboring molecules (figure 1). The change from dry to wet insulin then appears to involve an angular shift of the molecules from these close-packed positions. Further, the new peak positions in the wet (0001) projection are not far from a second hexagonal network, which might again bring the unit points into close contact.

It is interesting that a similar effect can be, very roughly, traced in the wet hemoglobin projection on (010). Nearly all the peaks present lie close to points on a network formed by intersection of the planes (006) and $\overline{(1201)}$ (figure 9). Bernal's procedure would lead one to deduce that here again the molecular pattern was roughly hexagonal in projection and formed by thirty-six points about the origin,—significantly double the number in insulin. Counting the origin peaks, the structures in insulin and hemoglobin would consist of nineteen and thirty-seven subgroups in projection, respectively.

Whatever is the interpretation of these effects, it is certain that the $\overline{(1201)}$ plane is important in the hemoglobin crystal structure. The two greater refractive indices of the crystal lie parallel to this plane, and also probably, from the examination of the absorption spectra (6), the heme groups of hemoglobin.

There are considerable difficulties in seeking an explanation of these Patterson projections, particularly in the more complicated structures such as lactoglobulin and hemoglobin. In these cases every maximum in the Patterson projection may correspond either to an intermolecular or

to an intramolecular vector and probably to both. The case of insulin is simpler, since the individual maxima must represent intramolecular vectors, the only doubt being with which molecule and with how many molecules in the lattice they are associated. It seems possible at first sight to make the distinction by comparing the wet and dry (0001) patterns from insulin. Here one would naturally associate the vectors that appear to move together with intramolecular vectors within one molecule, and this assumption has, in fact, underlain much of the previous discussion. It is clear that the peak movement is such that it tends to bring the peaks associated by Dr. Wrinch as due to vectors within one molecule out of co-ordination with one another. Other assumptions than simple movement would therefore have to be introduced to relate the Wrinch structure to

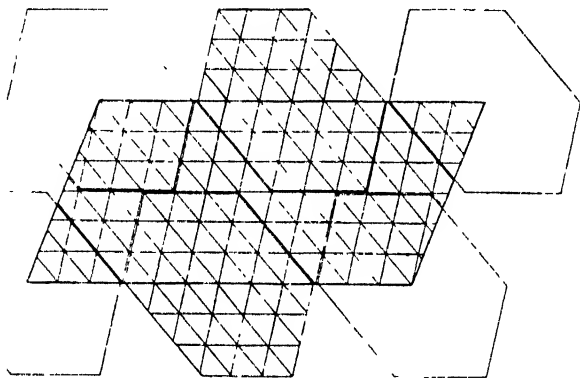


FIG. 9 Hemoglobin Idealized picture of the xz projection, showing the network of points with the outlines of six intramolecular patterns. The distances apart of the intersections of the network are 8.6, 9.1, and 11 Å. The two central hexagons are at $z = \frac{1}{2}$, the others at $z = 1$.

the insulin patterns. It is reasonable to compare with this conclusion the effects to be expected with the model proposed by Bernal for insulin. Bernal has suggested that the molecules can be considered as consisting of a number of subgroups arranged in cubic close-packing. In projection the point-group associated with this structure is shown in figure 10, together with that on which the Wrinch interpretation is based, for comparison. The Patterson projection derived from the Bernal arrangement⁴ is one in which all the peaks are complex and include three or four intramolecular vectors which coincide in the pattern from air-dried insulin,

⁴ Examination of figure 10 will show that the two groups at 3 and 3' in the Bernal structure are not at the same z level. The criticism of this theory by Wrinch and Langmuir (Proc. Phys. Soc. (London) **51**, 617 (1939)) is therefore not valid.

owing to the close-packed arrangement described above. The short vectors within one molecule contribute most to the peak intensity. On turning the molecule out of the close-packed positions one would expect the movement to be reflected in the peak positions, but these should also be considerably blurred if the movement is not exactly into the next close contact positions. Blurring certainly appears to be present, but not quite in the direction expected from simple theory. It is clear that in both cases it may be argued that deductions from the point-groups alone are oversimplified and that complete agreement will depend on the molecular model adopted.

The two theories are derived from quite different molecular models. According to the Wrinch theory, the insulin molecule is a single polyhedron; in the Bernal theory it consists of subgroups of twelve or twenty-

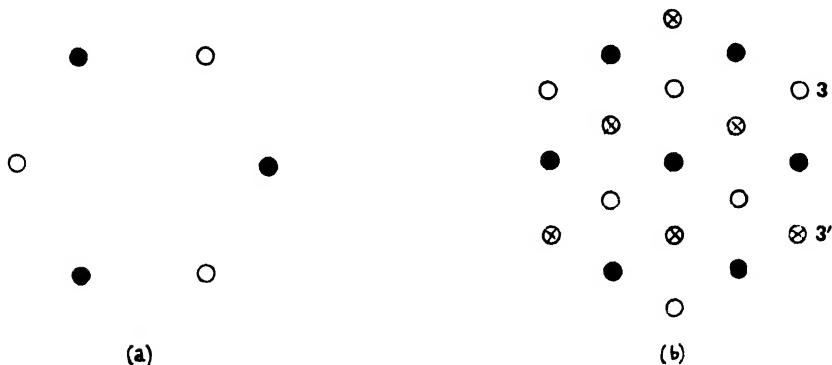


FIG. 10. Insulin: (a) Wrinch point group, (b) Bernal point group. The points at different heights are indicated by the variation in shading.

four amino-acid residues which are arranged in cubic close-packing, and probably linked through R groups such as cystine or glutamic acid. The amino-acid residues in each subgroup are linked in a chain and can be ordered in several ways, e.g., to cover the surface of a cube. Other polyhedra midway in size between these two have been devised by Talmud (7), and certainly there are quite different possibilities. It is clear from the existing controversy that no theory so far put forward is completely convincing; and in conclusion I should like to emphasize two points in the insulin data that seem important.

First, as an experimentalist, I should prefer to associate angular shift of the Patterson peaks for wet and dry insulin with that of the molecule.

Secondly, I am strongly of the opinion that the peak arrangement in air-dried insulin and its relation to close-packing structures is no accident,

but is an expression of some characteristic of the actual protein structure, whether this has the form discussed by Bernal or one completely different.

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COORDINATION COMPOUNDS OF OLEFINS WITH METALLIC SALTS

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Received December 13, 1940

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I. INTRODUCTION

The metal-olefin compounds have received considerable attention recently,—in part because of their practical importance, but primarily because of the many challenging problems centering around their structure. From the time the very first of these compounds was prepared, the mode of linkage of the double bond to the metal has been the subject of much speculation. While the ability to combine with olefins is quite widely distributed among the metals, it is most strongly exhibited by platinum. The formation of such compounds is not limited to hydrocarbons alone. Analogous substances containing unsaturated alcohols, acids, aldehydes, and esters are known, and in each of these the unsaturated molecule seems to occupy only one coordination position in the complex.

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II. HISTORICAL

A. COMPOUNDS OF THE GROUP VIII METALS

1. *Platinum olefin compounds*

The credit for preparing the first compound of this type is to be given, perhaps, to Zeise, who in 1827 published a note concerning the research which was being done on platinum compounds in his laboratory (125). Three years later this work was published in Latin (126), and the following year a condensed form appeared in German (127). Berzelius (8) in 1830, however, had reported that by refluxing a mixture of alcohol and sodium chloroplatinate a very acid solution was obtained, which, when concentrated with potassium chloride, yielded yellow crystals. Qualitative analysis of this substance indicated it to be a double salt of potassium chloride and platinumous chloride, along with an "atherartigen Substanz." When Zeise's investigation appeared shortly thereafter, Berzelius concluded (9) that his product was identical with that obtained by Zeise.

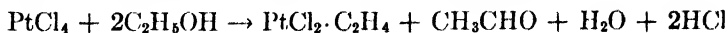
Zeise discovered that when platinic chloride was boiled with alcohol an acid solution resulted. By treating this solution with potassium chloride he obtained a compound in which potassium, platinum, chlorine, and the constituents of ethylene were present. The composition of the anhydrous substance was indicated to be $KCl\ PtCl_2 \cdot C_2H_4$. These analyses were immediately challenged by Liebig (70), who maintained that the anhydrous salt still contained the components of one-half a molecule of water for the simplest formula. He thought that the radical $C_4H_{10}O$ was present and that the correct formulation of the compound should be $2KCl \cdot 2PtCl_2 \cdot C_4H_{10}O$. Repetition of the analysis by Zeise (128) simply served to confirm his original contention that ethylene was present.

Zeise considered that he had obtained the basic compound of this series of substances, $PtCl_2 \cdot C_2H_4$, by treating the ammonium salt (prepared similarly to the potassium salt) with chloroplatinic acid. His product, however, was probably the more or less decomposed acid $H[PtCl_3 \cdot C_2H_4]$, now generally referred to as "Zeise's acid." The potassium and ammonium salts he obtained were probably $K[PtCl_3 \cdot C_2H_4] \cdot H_2O$ and $NH_4[PtCl_3 \cdot C_2H_4] \cdot H_2O$. Zeise apparently also prepared the non-ionic compound $[PtCl_2 \cdot NH_3 \cdot C_2H_4]$.

Griess and Martius (32), in 1861, not only confirmed Zeise's formula but also demonstrated that ethylene was liberated when Zeise's compound was thermally decomposed. These workers also prepared compounds analogous to Zeise's salt but containing aniline hydrochloride, diphenylamine hydrochloride, and ethylenediamine hydrochloride, instead of ammonium chloride and potassium chloride. The structures which Griess and Martius proposed for these compounds are mainly of historical interest at present.

Hoping to settle conclusively the question as to whether these substances actually contained ethylene, Birnbaum (13) undertook to synthesize such compounds by a method different from any heretofore employed. The reaction of alcohol on platinic chloride is apparently quite a complicated process, as evidenced by the great variety of products obtained. For this reason Zeise was unable to clarify completely the course of the reaction. Birnbaum contended that the fact that Griess and Martius had observed ethylene as one of the decomposition products of Zeise's salt was not proof that ethylene as such was present in the molecule originally; it might be simply a secondary decomposition product. This seemed even more plausible when it was considered that the thermal decomposition of the salt gave not only platinous chloride and ethylene but also an appreciable quantity of metallic platinum and carbonaceous decomposition products. Birnbaum reasoned that if the complexes could be synthesized from ethylene as a starting material, all ambiguities as to the resulting structure would be eliminated. He succeeded in making not only ethylene but also several of its homologs combine with platinous chloride dissolved in hydrochloric acid. Treatment of the resulting solution with potassium chloride gave a substance which proved to be Zeise's salt, $\text{KCl} \cdot \text{PtCl}_2 \cdot \text{C}_2\text{H}_4 \cdot \text{H}_2\text{O}$. The corresponding propylene compound was obtained in the same way. Amylene was found to react under similar conditions, but the compound formed was very unstable. The amylene compound was also obtained by employing Zeise's method, i.e., refluxing amyl alcohol with platinic chloride. The results indicated to Birnbaum that the coordinating abilities of propylene and amylene are certainly less than that of ethylene.

Since an aldehyde was detected as one of the products of the reaction of an alcohol with platinic chloride, Birnbaum proposed the following equation as the probable course of the primary reaction:



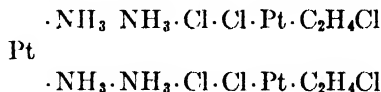
Zeise (129) had also demonstrated the presence of an aldehyde in his distillate. Moreover, he had isolated ethyl chloride, which was formed as a secondary product, and literally expressed the above equation in words.

An attempt by Birnbaum to prepare Zeise's salt by the reaction of ethylene on a potassium chloroplatinite solution was unsuccessful. When platinic chloride was refluxed with methyl alcohol, partial reduction of the platinic chloride to platinous chloride appeared to take place, but no further reaction occurred.

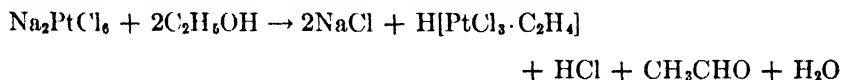
Using Zeise's original method, Chojnacki (18) prepared the ethylene-platinous bromide compound $\text{KBr} \cdot \text{PtBr}_2 \cdot \text{C}_2\text{H}_4 \cdot \text{H}_2\text{O}$, which was entirely analogous to Zeise's salt

; Jorgensen (61) sought to improve the yields of these olefin compounds

by a modification of Berzelius' method (8). Apparently only one attempt (96) had been made to repeat Berzelius' experiment, and this had resulted in failure. Jorgensen found that the reaction was slow and that refluxing with absolute alcohol for about 10 hr. was necessary. The solution became acidic and some reduction to metallic platinum occurred. The very soluble potassium salt of Zeise's acid could be obtained from the resulting yellow solution, but Jorgensen more conveniently isolated the acid as its tetrammine platinous salt, the formula of which he indicated as



The primary reaction for this method was given as follows:



Biilmann (10) demonstrated that the same general type of compound can be made from an unsaturated alcohol. Allyl alcohol reacted readily with a warm aqueous solution of potassium chloroplatinite according to the equation



This alcohol did not react, however, with the acid H_2PtCl_4 . The compound $\text{K}[\text{PtBr}_3 \cdot \text{C}_3\text{H}_5\text{OH}]$ was obtained in a strictly analogous manner (11) from potassium bromoplatinite.

Unsaturated acids were found (12) to form complex compounds with platinum, according to the general reaction²



Interestingly enough, acids with the double bond in the α -position with respect to the carboxyl group did not form complexes; however, acids in which the double bonds were in the β -position or farther removed from the carboxyl group formed complexes, although it was observed that the rate of reaction of the latter class was slower than with allyl alcohol.

When a suspension of platinous chloride in trimethylethylene is saturated with gaseous hydrogen chloride, a brown liquid is obtained from which a platinous chloride trimethylethylene complex, $2\text{PtCl}_2 \cdot \text{C}_6\text{H}_{10}$, may be isolated (68). The reaction is slow. The true molecular weight of the substance was not determined.

A number of compounds analogous to Zeise's salt, containing unsatu-

² Un = olefinic molecule.

rated acids, esters, alcohols, and aldehydes, were obtained from potassium chloroplatinite by Pfeiffer and Hoyer (94). The course of these reactions was followed by a color change of the solution from red to yellow as the potassium chloroplatinite was converted into the complex. Inasmuch as Pfeiffer and Hoyer were unsuccessful in obtaining complexes of olefinic substances with aluminum bromide, stannic chloride, etc., they suggested that the olefin bond might conceivably be a specific coördinating group for platinous salts.

Anderson (3) succeeded in isolating the primary member of the ethylene series, $\text{PtCl}_2 \cdot \text{C}_2\text{H}_4$, from the solution obtained by reducing sodium chloroplatinate with alcohol by evaporation below 50°C . in a high vacuum. A brown, tarry, strongly acid mass was obtained, from which the ethylene-platinous chloride was separated by extraction with chloroform, followed by recrystallization from benzene. The analogous compound, styrene-platinous chloride, $\text{PtCl}_2 \cdot \text{C}_6\text{H}_5\text{CH}=\text{CH}_2$, was also obtained (4). A significant reaction was utilized in this preparation styrene was found to displace ethylene quantitatively from $\text{PtCl}_2 \cdot \text{C}_2\text{H}_4$ in a vacuum at room temperature. Employing the same method, Anderson readily obtained potassium styrenetrichloroplatinite, $\text{K}[\text{PtCl}_3 \cdot \text{C}_6\text{H}_5\text{CH}=\text{CH}_2]$, from Zeise's salt. The corresponding indene compound was found to be much less stable, and while the cyclohexene compound could not be isolated, its formation was indicated. The only compounds obtained which were comparable in stability to Zeise's ethylene compounds were styrene-platinous chloride and potassium styrenetrichloroplatinite.

As a result of this systematic investigation, Anderson drew the interesting conclusion that the coordinating abilities of the olefins decrease in the order $\text{CH}_2=\text{CH}_2 > \text{C}_6\text{H}_5\text{CH}=\text{CH}_2 > \text{indene} > \text{cyclohexene} >$

C_2H_5
 $(\text{C}_6\text{H}_5)_2\text{C}=\text{CH}_2$ and $\begin{array}{c} \diagup \\ \text{C}=\text{CH}_2 \\ \diagdown \\ \text{CH}_3 \end{array}$. Although no pure compounds of the

type $\text{PtCl}_2 \cdot 2\text{C}_6\text{H}_5\text{CH}=\text{CH}_2$ could be isolated, Anderson found evidence for their existence.

A somewhat radical departure was made by Kharasch and Ashford (64) in the method of preparing compounds of the type $\text{PtCl}_2 \cdot \text{Un}$. Anhydrous platinic chloride or bromide was treated in an anhydrous solvent with the olefinic substance. The method is a rapid one and seems quite general. The reactions involved are not well understood and are probably complex. Hydrogen halide is usually evolved during the reaction, and in some instances a small quantity of platinum separates. Halogenation of the unsaturated compound may also occur. As pointed out by Kharasch and Ashford, the most direct method would be to combine a platinous halide

with an unsaturated compound, but attempts to apply this method have been less satisfactory, owing to the insolubility and inertness of these platinous compounds.

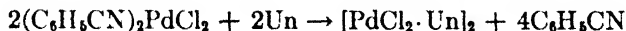
The method of Kharasch and Ashford gave compounds with chloro-substituted olefins but did not seem applicable to unsaturated acids and their esters. It is interesting to note that *trans*-dichloroethylene and *trans*-diphenylethylene formed crystalline coordination compounds with platinum, whereas the corresponding *cis*-isomers failed to react.

Chernyaev and Hel'man (17, 37) prepared Zeise's salt by passing ethylene for 15 days through a concentrated aqueous solution of potassium chloroplatinite containing 3 to 5 per cent of hydrochloric acid, precipitating the tetramine salt, $[\text{Pt}(\text{NH}_3)_4][\text{PtCl}_3 \cdot \text{C}_2\text{H}_4]_2$, with aqueous $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2$, and regenerating $\text{K}[\text{PtCl}_3 \cdot \text{C}_2\text{H}_4]$ with potassium chloroplatinite. Compounds of the general formula $[\text{PtRX}_2 \cdot \text{C}_2\text{H}_4]$ were also prepared. In this series R equals thiourea, ammonia, pyridine, and quinoline, and X equals CN, NCS, NO_2 , I, Br, and Cl.

Hel'man examined (39) the interesting question as to whether the presence of two double bonds, as in butadiene, makes it possible for such a substance to occupy two coordination positions and thus form a five-membered ring. The evidence seems to indicate that no ring is formed and that each double bond functions separately. Only one coordination position is therefore taken up in such compounds as $[\text{PtCl}_2 \cdot \text{C}_5\text{H}_5\text{N} \cdot \text{C}_4\text{H}_6]$ and $[\text{PtCl}_2 \cdot \text{NH}_3 \cdot \text{C}_4\text{H}_6]$.

2. Palladium-olefin compounds

The palladium olefin compound $\text{PdCl}_2 \cdot \text{C}_5\text{H}_{10}$ was reported (68) to be formed when palladous chloride, trimethylethylene, and a trace of some basic substance were allowed to react in a closed tube at room temperature. Kharasch, Seyler, and Mayo (65) were completely unsuccessful in their attempts to prepare the same and analogous compounds by this method. A quite different approach to the problem by these workers, however, resulted in the development of a very effective and general method for the preparation of these compounds. Palladous chloride was converted into the dibenzonitrile-palladous chloride derivative, which, strikingly enough, reacted very rapidly at room temperature with olefins to yield the desired derivatives. The steps in such a preparation are indicated below:³



³ See page 249 for the proof of the dimeric structure of $[\text{PdCl}_2 \cdot \text{Un}]_2$.

The displacement of the benzonitrile was thought to take place practically quantitatively, but, because of the experimental difficulties involved, the yields seldom exceeded 70 per cent. By the use of this method compounds containing ethylene, isobutylene, cyclohexene, and styrene were obtained.

3. Iron-olefin compounds

Kachler (62) reported the preparation of the compound $\text{FeCl}_2 \cdot \text{C}_2\text{H}_4 \cdot 2\text{H}_2\text{O}$ and pointed out that it corresponds to the ethylene-platinous chloride compound $\text{PtCl}_2 \cdot \text{C}_2\text{H}_4$. This substance was said to form when ferric chloride in ether in the presence of a small quantity of phosphorus was heated in a sealed tube. The following equation was given for the reaction:



Efforts to prepare the compound from alcohol instead of ether were not successful.

An attempt was made by Chojnacki (18) to prepare the compound reported by Kachler, starting with ferrous chloride and ethylene. This corresponds to the method used by Birnbaum in preparing ethylene-platinous chloride from ethylene and platinous chloride. Chojnacki was not successful in this, but claimed that he did prepare the corresponding ethylene-ferrous bromide compound whose composition he gave as $\text{C}_2\text{H}_4 \cdot \text{FeBr}_2 \cdot 2\text{H}_2\text{O}$. He also stated that a solution of this compound, when treated with a concentrated solution of potassium bromide and evaporated slowly, deposited almost colorless crystals which upon analysis were shown to contain iron, bromine, potassium, and ethylene. No formula was advanced for this substance.

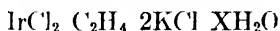
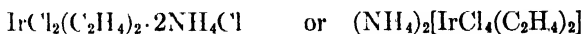
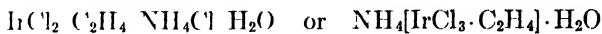
Manchot and Haas (75) were unable to repeat the work of Kachler and Chojnacki. They claimed that Kachler's supposed ethylene ferrous chloride was really a partially decomposed ether addition compound.

The long heating of iron pentacarbonyl with butadiene was reported (97) to give the complex $\text{Fe}(\text{CO})_3 \cdot \text{C}_4\text{H}_6$, in which two of the five carbon monoxide molecules of the carbonyl had been replaced by one molecule of butadiene. The unsaturated hydrocarbon occupies one or two coördination positions of the iron atom. Less well-defined compounds approximating to $\text{Fe}(\text{CO})_3 \cdot (\text{C}_6\text{H}_8)_3$, $\text{Fe}(\text{CO})_3 \cdot (\text{C}_6\text{H}_8)_2$, and $\text{Fe}(\text{CO})_3 \cdot (\text{C}_6\text{H}_{10})_2$ were obtained with isoprene and with β, γ -dimethylbutadiene (also see page 236).

4. Iridium-olefin compounds

Sadtler (100) reported the preparation of the iridium chloride-ethylene complex $\text{IrCl}_2 \cdot \text{C}_2\text{H}_4$ by the treatment of iridic chloride with absolute

alcohol. The equation for the reaction was said to be similar to that for the preparation of the corresponding platinum compound. When the resulting solution was treated with potassium chloride or ammonium chloride, mixtures of substances resulted. The following complexes were said to have been identified in these mixtures:



No compounds of iridium could be obtained by passing ethylene through an iridium chloride solution, nor did a reaction occur when iridium chloride was treated directly with ethylene.

If the above complexes are true chemical individuals, the compound $(\text{NH}_4)_2[\text{IrCl}_4(\text{C}_2\text{H}_4)_2]$ has no counterpart in the platinum series. However, the above experimental work does not appear convincing, and these substances would bear further investigation.

B COMPOUNDS OF OTHER METALS

1. Aluminum-olefin compounds

The product obtained by cracking petroleum with aluminum chloride under certain conditions consists largely of saturated hydrocarbons. On the theory that the aluminum chloride combines with any unsaturated hydrocarbons formed and holds them back from the distillate, Henderson and Gangloff (42) studied the reaction of aluminum chloride with various unsaturated molecules. Direct reaction of the unsaturated molecule with anhydrous aluminum chloride proved to be unsatisfactory as a means of obtaining compounds which approached definite compositions. A fairly nice crystalline product was obtained from a solution of aluminum chloride in absolute alcohol into which ethylene was passed. This substance was extremely difficult to analyze because of its instability and hygroscopic character, but seemed to correspond to the formula $\text{AlCl}_3 \cdot 3\text{C}_2\text{H}_4 \cdot \text{H}_2\text{O}$. Later (27) a second product was obtained with ethylene, which appeared to be either $\text{AlCl}_3 \cdot 3\text{C}_2\text{H}_4 \cdot 2\text{H}_2\text{O}$ or $\text{AlCl}_3 \cdot \text{C}_2\text{H}_4 \cdot 2\text{C}_2\text{H}_5\text{OH}$. In a similar way these investigators were able to prepare compounds of aluminum chloride with other unsaturated hydrocarbons as well as with unsaturated acids, aldehydes, and alcohols.

Very similar results were obtained with ferric chloride, although the reactions were less vigorous and not so complete. Ferric chloride and amylene in methyl alcohol gave $\text{FeCl}_3 \cdot \text{C}_6\text{H}_{10} \cdot \text{CH}_3\text{OH}$.

From the results of a study of the reactions of aluminum chloride and

unsaturated hydrocarbons, Stanley (111) concluded that any explanation for such reactions must take into account the formation of an aluminum chloride-hydrocarbon complex. Results of an earlier study led Szayna (115) to somewhat similar conclusions.

2. Zinc-olefin compounds

There seems to be some evidence (67, 68) for the existence of addition compounds of zinc chloride and amylene, various formulas having been reported, such as $\text{ZnCl}_2 \cdot \text{C}_5\text{H}_{10}$ and $2\text{ZnCl}_2 \cdot \text{C}_5\text{H}_{10}$. These apparently have been little studied and their exact nature is not clear.

3. Copper-olefin compounds

Berthelot (7) observed that a hydrochloric acid solution of cuprous chloride absorbed ethylene to the extent of 0.17 mole ethylene to 1 mole of cuprous chloride. Propylene was absorbed to a slightly greater extent, the ratio being 0.25 to 1.

It was reported by Manchot and Brandt (74) that cuprous chloride and ethylene combine to form an unstable compound in which 1 mole of ethylene unites with 1 mole of cuprous chloride. The combination did not occur in the absence of water. The compound was too unstable to isolate, and these investigators considered that the combination between these two molecules occurs not through specific atoms but through "latent affinities" of the entire molecule.

Ethylene under pressure was found to give with *solid* cuprous chloride an addition compound (117) which contained 1 mole of ethylene to 1 mole of cuprous chloride, $\text{CuCl} \cdot \text{C}_2\text{H}_4$. It is interesting to note in this connection that no polymerization of ethylene was observed in the presence of cuprous chloride at pressures of 65 to 80 atmospheres and temperatures of 100 to 200°C.

Later (30) it was shown that such a reaction is not limited to ethylene and cuprous chloride. Ethylene, propylene, and isobutylene are absorbed by solid cuprous chloride, and ethylene is absorbed by cuprous bromide. Under suitable conditions olefins may be recovered from gaseous or liquid mixtures by the use of solid cuprous halides.

Cuprous chloride also forms a complex with butadiene (72). This is a rather stable pale yellow powder having either the composition $\text{Cu}_2\text{Cl}_2 \cdot \text{C}_4\text{H}_6 \cdot 4\text{H}_2\text{O}$ or $\text{Cu}_2\text{Cl}_2 \cdot \text{C}_4\text{H}_6$, depending upon the conditions under which it is prepared.

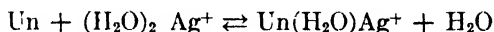
4. Silver-olefin compounds

By means of a distribution method, Winstein and Lucas (123) have studied the coördination complexes which the silver ion forms with various olefinic substances. The method consists essentially in distributing the

unsaturated compound between carbon tetrachloride and aqueous silver nitrate, or between carbon tetrachloride and mixtures of silver nitrate and potassium nitrate, and comparing the quantities of the organic substance dissolved in the aqueous layer under these circumstances with the quantity dissolved in the aqueous layer when the latter contains only potassium nitrate.

The unsaturated compounds used were certain monoolefins, diolefins, and unsaturated oxygenated compounds, such as allyl alcohol and crotonaldehyde. The reaction to form a complex was found to be rapid and reversible. Three types of complexes were observed: (1) combination of one silver ion with one unsaturated molecule, (2) combination of two silver ions with one unsaturated molecule, and (3) combination of one silver ion with two unsaturated molecules. In most cases only solutions of these complexes were obtained, although in the cases of two hydrocarbons solid silver complexes were isolated.

According to Winstein and Lucas, probably the first step in the chemical change which takes place when a silver-olefin complex is formed is the replacement of a coordinated water molecule by the ethylenic molecule, as follows.



Therefore, it follows that if the strength of the coordinate bond holding a water molecule is much greater than the strength of the silver-olefin bond, there will be little or no tendency for the olefinic substance to form a complex with the silver ion. Of the ten metallic ions studied in this work, silver ion was the only one which entered into complex formation. In aqueous solution the following ions failed to form complexes with an olefin: Cd^{++} , Co^{++} , Cr^{+++} , Cu^{++} , Fe^{+++} , Ni^{++} , Pb^{++} , Tl^+ , and Zn^{++} .

5. Mercury-olefin compounds

The compounds of olefinic substances with mercuric salts have been more extensively studied than any other class of olefin-inorganic salt complexes. These compounds seem for the most part to be of a somewhat different type⁴ from those of platinum, palladium, silver, etc.

Mercury compounds have been made containing ethylene and its homologs, both aliphatic and aromatic unsaturated alcohols, and unsaturated acids. The gaseous olefins react readily with mercuric salts to form compounds (20, 46, 102, 103) from which the olefins may be regenerated by the action of hydrochloric acid. The corresponding reaction of the higher olefins is frequently accompanied by reduction of the mercury to the mercurous state with simultaneous oxidation of the hydrocarbon. If alcoholic

⁴ The structures of the mercury-olefin compounds are discussed on page 258.

solvents are employed, the solvent sometimes enters into the reaction and the final products then contain alkoxy groups. Tausz (116) has described compounds of mercuric acetate with cyclohexene, methylenecyclohexene, and the terpenes. He also prepared double compounds of amylene and hexene. Hugel and Hibou (50) have reported that for each olefin the addition compounds with mercuric salts have variable compositions depending on the experimental conditions.

As a class, the mercury-olefin compounds are useful as intermediates in organic syntheses.

6. Miscellaneous compounds

In connection with the compounds of olefins with inorganic salts, the work of Hofmann and von Narbutt (47) should be mentioned. These investigators reported an unusual reaction which appears to involve direct addition across the double bond. The reaction between dicyclopentadiene and potassium chloroplatinite in propyl alcohol yielded a product, $\text{PtCl}_2 \cdot \text{C}_{10}\text{H}_{12}$, which probably should be formulated as $\text{PtCl} \cdot \text{C}_{10}\text{H}_{12}\text{Cl}$. This compound is said to differ very markedly (4) in properties from the ethylene- and styrene-platinous chlorides.

Other unsaturated hydrocarbon-metallic salt complexes have been reported. These do not fall within the province of this survey and will therefore be considered only very briefly. Acetylene complexes of aluminum and copper salts are known. Typical of these are the following. $\text{AlCl}_3 \cdot 3\text{C}_2\text{H}_2 \cdot \text{H}_2\text{O}$ (42), $2\text{CuCl} \cdot \text{C}_2\text{H}_2$, and $\text{KCl} \cdot 4\text{CuCl} \cdot \text{C}_2\text{H}_2$ (121). A number of complexes of aluminum chloride and aluminum bromide with benzene and substituted benzenes have been prepared. Of these the following are representative: $\text{AlCl}_3 \cdot 3\text{C}_6\text{H}_6$ (121), $\text{Al}_2\text{Cl}_6 \cdot 2\text{sym-C}_6\text{H}_3(\text{C}_2\text{H}_5)_3 \cdot \text{HCl}$, $\text{Al}_2\text{Br}_6 \cdot \text{sym-C}_6\text{H}_3(\text{C}_2\text{H}_5)_3$, and $\text{Al}_2\text{Br}_6 \cdot \text{C}_6\text{H}_5\text{CH}_3$ (84). Investigations seem to indicate that the stability of such complexes increases as the number of alkyl groups present increases. Antimony pentachloride forms compounds with several aromatic hydrocarbons; the reaction with anthracene to give an intensely green precipitate has been suggested as a qualitative means of identifying this hydrocarbon (43). Antimony trichloride is reported (99) to form the complex $3\text{SbCl}_3 \cdot \text{C}_6\text{H}_6$. The compound $\text{Ni}(\text{CN})_2 \cdot \text{NH}_3 \cdot \text{C}_6\text{H}_6$ results (45) when a strongly ammoniacal solution of nickelous cyanide is shaken with benzene. Similar compounds containing phenol, aniline, thiophene, and pyrrole instead of benzene have also been obtained (44).

III. DISCUSSION OF METHODS OF PREPARING METAL-OLEFIN COMPOUNDS

From the foregoing survey it is evident that most of the satisfactory methods for the preparation and isolation of metallic salt-olefin complexes are concerned with platinum and palladium compounds. For this reason

the following discussion of the relative merits of the more important preparatory methods is confined to the compounds of these two metals.

Method A —The reduction of platinic chloride by a saturated primary alcohol (Zeise's original method) seems to involve many side reactions, and the yields of the corresponding olefinic complex are never high.

Method B —The reaction in which sodium chloroplatinate is reduced by a saturated primary alcohol likewise is very complex and is none too satisfactory for other than ethyl alcohol.

Both methods A and B give, obviously, only the α -unsaturated complexes.

Method C —In reactions of olefinic compounds with potassium chloroplatinite in aqueous alcohol solution, the unsaturated molecule enters the coordination sphere and displaces one chloride ion:



This displacement proceeds at room temperature or slightly above but is slow, owing to the insolubility of the chloroplatinite in alcohol. Reactions of this type are reversible (4), and in carrying out preparations by this method an equilibrium must be attained. Zeise's salt is stable enough to be recrystallized unchanged in the presence of an excess of potassium chloride or hydrochloric acid, while with other olefin complexes under the same conditions recrystallization may lead only to potassium chloroplatinite (13). Anderson points out that the extent to which the olefin-containing complex anion is formed in this reaction may thus afford some qualitative measure of the coordinating power of the olefin.

Method D.—In some cases olefins add directly to platinous chloride dissolved in an absolute alcohol hydrochloric acid mixture (4). Since the system is homogeneous, the reaction proceeds quite rapidly but usually is incomplete, owing to the presence of the necessarily high concentration of hydrochloric acid.

Method E.—A significant method of preparation is by the direct replacement of one olefin by another either under reduced pressure or in solution.

- (1) Under reduced pressure: As Anderson points out (4), the factors involved here are the relative stabilities of the salts, i.e., the relative coordinating tendencies of the olefins, and the relative volatilities of the olefins under reduced pressure. Styrene readily displaces ethylene from Zeise's salt, yet the styrene complex is definitely less stable than the ethylene salt.

- (2) In solution: In some cases this method might be of some importance. It was found to be applicable to the palladium olefin compounds (65), but as a means of preparing these complexes was of only secondary importance.

Method F.—Undoubtedly the most satisfactory method of preparing complexes of the type $[\text{PtX}_2 \cdot \text{Un}]_2$ is the method in which the platinum halide is treated in an anhydrous solvent, such as benzene or glacial acetic acid, with the unsaturated compound. The reaction proceeds smoothly and good yields are obtained. In general, platinum bromide is somewhat better for these reactions, probably because the bromide is more soluble than the chloride.

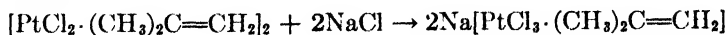
Method G.—Palladous chloride olefin derivatives can be prepared by the action of the olefin on dibenzonitrile-palladous chloride. The reaction takes place readily and gives good yields.

It is interesting to note in this connection that while dibenzonitrile-palladous chloride reacts with olefins to give compounds of the type $[\text{PdCl}_2 \cdot \text{Un}]_2$, the platinumous chloride compounds $(\text{RCN})_2 \cdot \text{PtCl}_2$ do not react with olefins. As a matter of fact, the nitrile-platinumous chloride compounds can be formed from the olefin derivatives and a nitrile.

IV. PROPERTIES AND REACTIONS OF METAL-OLEFIN COMPOUNDS

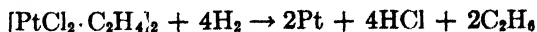
The coordination compounds $\text{PtCl}_2 \cdot \text{Un}$ (usually formulated as $[\text{PtCl}_2 \cdot \text{Un}]_2$)⁵ are well-defined crystalline substances. They are decomposed by water, but in general are soluble in ether, chloroform, alcohol, and acetone. They are difficultly soluble or insoluble in glacial acetic acid, and only moderately soluble in cold benzene. Most of these compounds do not possess sharp melting points but darken over a range of several degrees. Their stabilities vary widely. The compound obtained from *trans*-dichloroethylene decomposes in several days, while that from dipentene remains unchanged after standing in the air for ten months (64).

The ethylene complex $[\text{PtCl}_2 \cdot \text{C}_2\text{H}_4]_2$ (3) and the isobutylene complex $[\text{PtCl}_2 \cdot (\text{CH}_3)_2\text{C}=\text{CH}_2]_2$ (64) are said to dissolve in aqueous potassium chloride or sodium chloride, presumably forming compounds analogous to Zeise's salt:



The ethylene-platinum compound $[\text{PtCl}_2 \cdot \text{C}_2\text{H}_4]_2$ decomposes without melting and does not combine with a further molecule of ethylene. This is in contrast to the reaction of $\text{PtCl}_2 \cdot \text{CO}$, which with carbon monoxide readily forms $\text{PtCl}_2 \cdot (\text{CO})_2$.

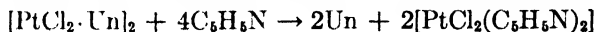
A further interesting and possibly significant reaction of the ethylene-platinumous chloride compound is the fact that it is rapidly and quantitatively reduced by hydrogen at room temperature to platinum, hydrogen chloride, and ethane (3):



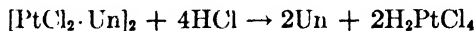
⁵ See page 249 for proof of dimeric structure.

The analogous styrene-platinous chloride, $[\text{PtCl}_2 \cdot \text{C}_6\text{H}_5\text{CH}=\text{CH}_2]_2$, is likewise reduced with extreme ease, reacting with hydrogen below 50°C . At higher temperatures incandescence may accompany the reaction (4).

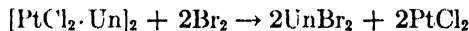
When these substances are treated with pyridine, decomposition of the complex occurs and the olefin and pyridine-platinous chloride result (64):



Concentrated hydrochloric acid causes decomposition in the following manner:



Bromine also decomposes these compounds; the liberated olefin in turn is brominated:



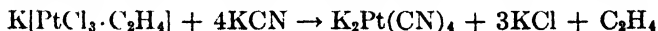
As previously mentioned, styrene and amylene can displace ethylene from $[\text{PtCl}_2 \cdot \text{C}_2\text{H}_4]_2$ at room temperature under reduced pressure (4).

The potassium salts $\text{K}[\text{PtCl}_3 \cdot \text{Un}]$ are usually too soluble to be obtained in the pure state and are more conveniently isolated as salts of $[\text{Pt}(\text{NH}_3)_4]^{++}$, strychnine, brucine, $[\text{Coen}_2(\text{C}_2\text{O}_4)]^+$, and *trans*- $[\text{Coen}_2\text{Cl}_2]^+$. The last two ions are in general more satisfactory than $[\text{Pt}(\text{NH}_3)_4]^{++}$ in forming well-crystallized and difficultly soluble salts of $[\text{PtCl}_3 \cdot \text{Un}]^-$. According to Anderson (3), the quinolinium salts of the olefintrichloroplatinates are characterized by relatively high stability and low solubility. Quinolinium ethylenetrichloroplatinite is sufficiently stable to be recrystallized from boiling water; in the presence of one molecular quantity of quinoline, it is converted into the non-electrolyte, quinoline-ethylene-platinous chloride:



Hot 2 *N* hydrochloric acid reverses this reaction.

Potassium cyanide effects the vigorous decomposition of $\text{K}[\text{PtCl}_3 \cdot \text{C}_2\text{H}_4]$, ethylene being liberated quantitatively according to the equation



Anderson points out (3) that other reagents capable of forming complex platinates, K_2PtX_4 , have a tendency to react similarly. However, the extent to which ethylene is displaced at room temperature depends on the nature of the complex. Anderson postulates that the reaction may proceed by an initial metathesis to a complex $\text{K}[\text{PtX}_3 \cdot \text{C}_2\text{H}_4]$, which may then break down spontaneously in the presence of an excess of reagent. When *X* equals *CN* this breakdown is immediate and quantitative; for *X* equals

NO_2 , however, only a fraction of the ethylene is displaced at room temperature. The evolution of ethylene with potassium thiocyanate is intermediate between that of cyanide and that of nitrite.

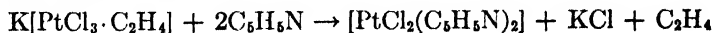
If ethylene-platinous chloride, $[\text{PtCl}_2 \cdot \text{C}_2\text{H}_4]_2$, is dissolved in hydrobromic acid and warmed, Chojnacki's acid, $\text{H}[\text{PtBr}_3 \cdot \text{C}_2\text{H}_4]$, is formed. This can be isolated as the quinolinium salt, which is decomposed by hot water and is consequently less stable than the corresponding chloro compound. From such considerations Anderson concludes that compounds of the general type $\text{K}[\text{PtX}_3 \cdot \text{C}_2\text{H}_4]$ appear to decrease in stability in the order $\text{X} = \text{Cl} > \text{Br} > \text{NO}_2 > \text{NCS} > \text{CN}$.

Above 90°C . Zeise's salt undergoes the following reaction with water (3):

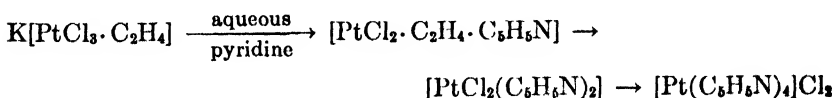


The corresponding styrene compound is somewhat less stable, dissociation occurs slowly at room temperature in aqueous solution, with the liberation of styrene and the deposition of platinous chloride.

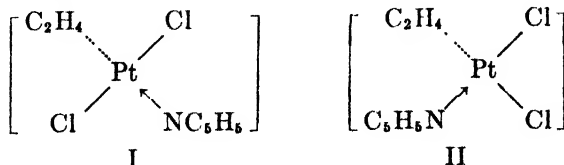
Ethylene is almost quantitatively expelled from Zeise's salt by anhydrous pyridine (3):



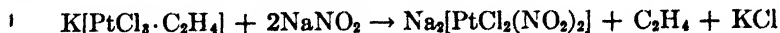
Excess aqueous pyridine ultimately transforms Zeise's salt into tetrapyridinoplatinous chloride:



The non-ionic complex $[\text{PtCl}_2 \cdot \text{C}_2\text{H}_4 \cdot \text{C}_5\text{H}_5\text{N}]$, which is the product of the first step in the above reaction, is thought to have a *trans*-configuration (formula I). Aqueous ammonia gives a similar compound. If, however, the compound $\text{K}[\text{PtCl}_3 \cdot \text{C}_5\text{H}_5\text{N}]$ is treated with ethylene, an isomer of the product of the above reaction is obtained (16, 17). This is presumably the *cis*-form (formula II).



When 1 mole of Zeise's salt reacts with 2 moles of sodium nitrite, ethylene splits out and $\text{Na}_2[\text{PtCl}_2(\text{NO}_2)_2]$ is formed (41):



If the proportions are mole for mole, the product is $\text{Na}[\text{PtCl}_2(\text{C}_2\text{H}_4)(\text{NO}_2)]$. This substance is very soluble but reacts with $[\text{Pt}(\text{NH}_3)_4]\text{Cl}_2$ to give $[\text{Pt}(\text{NH}_3)_4][\text{PtCl}_2(\text{C}_2\text{H}_4)(\text{NO}_2)]_2$. The C_2H_4 and NO_2 groups in this complex are said to be in the *trans*-positions.

Substitution about the double bond in olefinic substances seems to lower greatly the stability of the coordination compounds of the type $\text{K}[\text{PtCl}_3 \text{ Un}]$ (4). On the other hand, the water-solubility of the compounds derived from substituted olefins is higher than that of the ethylene compounds.

All of the palladium compounds obtained by Kharasch, Seyler, and Mayo (65) were colored, unstable, and rather insoluble in the common organic solvents. All were less stable in solution than when solid and were evidently less stable in acetone and alcohol than in other solvents. As a class, these compounds are less stable than the corresponding platinum compounds.

It might be expected that an olefin capable of forming a more stable compound would be able to displace the olefin from a less stable palladium-olefin complex. This appears to be the case (65). Cyclohexene formed the most stable compound and no other olefin was found to displace it from a solution of the cyclohexene-palladous chloride. The ethylene complex underwent displacement with cyclohexene but was not affected by styrene. The stabilities of the palladous chloride-olefin compounds in solution are therefore in the order cyclohexene > ethylene > styrene. The ethylene compound in the dry state apparently is less stable than the styrene compound, but this is believed to be due to the greater volatility of the ethylene.

Comparatively little information is available regarding the properties and reactions of other metallic salt olefin complexes. In most cases these substances are decidedly less stable than the platinum and palladium compounds and some of them are known only in solution.

V. TECHNICAL APPLICATIONS OF METAL-OLEFIN COMPOUNDS

There seems to be little doubt that the metallic salt olefin complexes are of great significance in many syntheses and processes involving olefinic substances. However, a scarcity of pure research and of pertinent journal and patent literature along this line makes it difficult even to speculate on the extent of the usefulness of these substances. Many compounds which are classed as catalysts for certain polymerization, hydration, and hydrogenation reactions may function by means of intermediate complex formation. The following brief survey indicates the general types of substances used in these and other reactions. Some are definitely known to form olefin complexes. Many of them, however, apparently have never been investigated from this standpoint.

The gaseous olefins are readily absorbed by aqueous solutions of cuprous, silver, mercuric, and platinous salts (26, 79). Complex compounds are probably formed. The olefins can be removed from these solutions by warming or by reducing the pressure. Scrubbing coal gas with an acid solution of silver nitrate under ordinary or increased pressures at 0–10°C. has been proposed (48, 49, 59, 114) as a method of removing ethylene and other olefins. These olefins may be subsequently recovered by heating the solution to 50–70°C. An ammoniacal solution of a cuprous salt was suggested (31, 60, 120) as a means of absorbing olefins (mainly ethylene) and carbon monoxide from coal gas, coke oven gases, and similar gases under 150 to 250 atmospheres pressure. The gases released from the ammoniacal solution may be scrubbed with either a solution of silver nitrate to absorb olefins or an ammoniacal solution of cuprous formate (5). Dubois (24) reported that both acid and ammoniacal solutions of cuprous chloride absorb olefins practically quantitatively from mixtures with other gases. Diolefins can be recovered from mixtures also containing monoolefins by taking advantage of the fact that the diolefins form insoluble complexes with certain heavy-metal salts (36). The more unsaturated constituents are removed from cracked oils by heating with ferric chloride solution (52), and hydrocarbon oil fractions of low boiling point are purified by treatment with aluminum chloride below 35°C. (113).

In the preparation of butadiene, advantage has been taken of the complex-forming ability of olefins (57). Butane is catalytically dehydrogenated to butylene. This is separated from the reaction mixture by solutions or suspensions of salts that absorb olefins, as, for example, silver nitrate, cupric nitrate, or mercurous nitrate. The butylene is easily recovered by heating the solution or reducing the pressure and is further dehydrogenated to butadiene. The latter can be removed by a solution of cuprous chloride containing ammonium chloride (72) or by other salts of the heavy metals of Groups I and II.

Of the many inorganic halides which have been found effective for condensing or polymerizing olefins, aluminum chloride is without doubt the most popular (81). The exact rôle that aluminum chloride plays in its reactions with olefins is uncertain. In the broadest sense it is looked upon as a catalyst. Olefins in the presence of aluminum chloride are said to polymerize, isomerize, cyclicize, and form paraffins and more highly unsaturated compounds (25).

Aluminum chloride has been used for converting gaseous and high-boiling olefins into low-boiling liquids (98), viscous oils (80, 106), synthetic lubricating oils (90), and synthetic resins (19). A patent (54) has been issued covering the process for preparing a double compound of ethylene and aluminum chloride. This compound is used for condensing hydro-

carbons. Aluminum fluoride has also found some application in these processes (56).

Boron halides, especially boron fluoride, have also been used extensively for polymerizing olefins. With boron fluoride, polymers of high molecular weight (86), rubber-like substances (21), and oils which compare favorably with petroleum lubricating oils (85) have been obtained. Boron halides are reported to form complexes with olefins; these complexes are useful in effecting the polymerization of olefins (28).

It seems not unlikely that the Friedel-Crafts reactions may proceed by means of an intermediate halide salt-complex. Aluminum chloride is the common catalyst for these reactions, but many other halides have been used (82).

Carbonyl compounds of metals such as tungsten, molybdenum, and those of Group VIII of the Periodic System have been used for converting high-boiling hydrocarbons into lower boiling forms by hydrogenation under pressure (55).

Metallic salt-olefin complexes are probably important intermediates in some stages of synthetic rubber manufacture, but, here again, detailed information is lacking. The polymerization of butadiene or of its derivatives is effected by boron fluoride (35), aluminum chloride (130), heavy-metal carbonyls such as those of iron, nickel, cobalt, chromium, etc. (2, 53), and by certain metal organic compounds, such as iron phthalocyanine sulfonic acid (58). Cuprous chloride solutions appear to be employed commercially (14, 66) to dimerize acetylene to vinylacetylene. This is the first step in making 2-chloro-1,3-butadiene, which in turn is readily polymerized to an excellent rubber.

The hydration of olefins in aqueous acid solutions is frequently greatly accelerated by the presence of certain metal salts. This may be another instance in which olefin complexes are important intermediates. Of the many compounds which have been useful in aiding these hydration reactions, compounds of the metals of Groups I, II, IV, V, and VIII are most frequently mentioned (23, 33, 78, 83, 109, 110, 119). Usually soluble catalytic salts such as sulfates, chlorides, and cyano complexes (77) are employed directly. In some cases, however, insoluble compounds may be added to the solution and then converted into soluble complexes by passing carbon monoxide or nitric oxide through the mixture (78).

VI. STRUCTURE OF METAL-OLEFIN COMPOUNDS

It seems evident that in a complex compound of an olefinic substance with a metallic salt the double bond is actually functioning in the metal-olefin union. Pfeiffer and Hoyer (94) arrived at this conclusion because they found that unsaturated alcohols, acids, and aldehydes form sub-

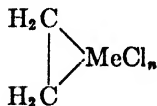
stances analogous to Zeise's salt, whereas the corresponding saturated compounds are completely incapable of reacting similarly. Winstein and Lucas (123), although admitting that the unsaturation of the coördinating molecule appears to be responsible for complex formation, point out that this interpretation is not entirely unambiguous, especially in those cases where the complex is composed of one metal ion and two unsaturated oxygenated molecules.

The interest and importance of this group of complexes center around the nature of the bond joining the metal and olefinic molecule. While these compounds differ in no marked way chemically from other types of complex salts (61), the mechanism of complex formation is obscure, in that the coördinating group possesses no "lone pair" of electrons with which to form the coördinate link.

The electron-pair theory is generally accepted as offering the best picture of the formation of a coördinate bond and the most satisfactory explanation of the many properties of coördination compounds. This view, however, is not universally accepted. Samuel and Hunter (101) point out that it is not altogether clear how a closed s^2 electron-pair can be shared with a second atom. They believe that the conception of the lone pair of electrons as an agent for true chemical linkage is not entirely in harmony with the results of band spectroscopy experiments. The acceptance of the electron-pair theory demands, in turn, the presence of a "lone pair" of electrons in order that an atom may exhibit "donor" functions. These are not apparent in the usual formulation of olefinic substances. In the opinion of some authorities the formation of complex compounds of the olefins with metallic salts supplies an interesting test of the application of the lone-pair bond theory.

Pfeiffer (91, 92) disposed of the structural problems associated with these compounds with the vague statement that the unsaturated carbon atoms of the olefinic molecule form the center of a region which, as a whole, is capable of exhibiting a kind of "secondary" valence. This so-called secondary valence may be satisfied by various ions or molecules. On this basis Zeise's salt is indicated as $K[C_2H_4 \dots PtCl_3]$.

Hantzsch (34) objected to this formulation on the ground that it represents carbon as having a coördination number of 5 instead of 4. To avoid this, he suggested a symmetrical structure (III) for these complexes:

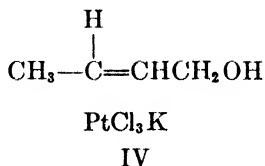


III

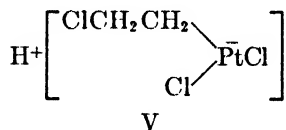
He entirely neglected, however, to elucidate the nature of the bonds between the hydrocarbon molecule and the metal.

Bülmann (10) suggested that with a properly substituted olefinic substance the potassium salt $K[PtCl_3 \cdot Un]$ might contain an asymmetric carbon atom; however, he believed that resolution was probably not possible because of the instability of the compound.

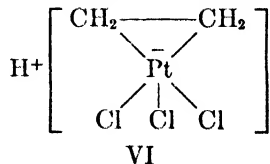
Pfeiffer and Hoyer (94) offered no explanation of the structure. They pointed out the possibility that an asymmetric carbon atom can be present in certain of these compounds and gave a formula as follows:



Drew, Pinkard, Wardlaw, and Cox (22) proposed the following structure for Zeise's acid:



In their opinion this compound is analogous to the corresponding ammonia and pyridine compounds, in which they believed that substantial evidence points to the presence of NH_3Cl and $\text{C}_5\text{H}_5\text{NCl}$ groups. They stated that in the case of Zeise's acid it is not possible, owing to the quadrivalency of carbon, to write the group ClCH_2CH_2 in any other manner, since the alternative formula would be that of a platinic instead of a platinous compound:

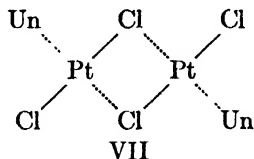


A considerable amount of evidence seems to justify the formulation of these compounds as derivatives of bivalent platinum. The more significant points are as follows: (1) Zeise's salt and its analogs can be prepared by direct reaction of the unsaturated substance on potassium chloroplatinite in aqueous alcohol solution (94). (2) Zeise's acid is formed by the direct absorption of ethylene by platinous chloride in alcoholic hydrogen chloride (13). (3) The reactions of these compounds with pyridine, hydrochloric acid, bromine, and potassium cyanide (pages 242-243)

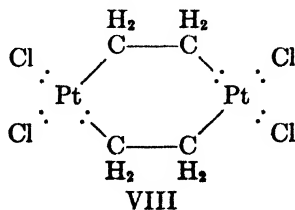
indicate the presence of platinous platinum. (4) The compound prepared from dipentene and platinic chloride, for example, is the same as the one obtained by treating dipentene with platinous chloride in the presence of dry hydrogen chloride (64).

The platinum-olefin and palladium-olefin compounds of the general type $MCl_2 \cdot Un$ probably possess a doubled molecular formula in order to maintain the coördination number of 4 for these metals. The coördination compounds of this series are unstable at higher temperatures, and consequently the ebullioscopic method for the determination of their molecular weights is not satisfactory. An approximate molecular weight determination, however, by the Barger-Rast method points to the dimeric form for ethylene-platinous chloride (3). Kharasch and Ashford report (64) that the solubility of isobutylene-platinous chloride in benzene permits an accurate determination of the molecular weight of this compound. Results indicate that it is bimolecular. Precise determinations of the molecular weights of the corresponding palladous-olefin compounds were not possible, but an approximate molecular weight of 409 was obtained for styrene-palladous chloride by the freezing-point method in benzene solution. This is intermediate between 282 for the monomeric and 564 for the dimeric form of $PdCl_2 \cdot C_6H_5CH=CH_2$.

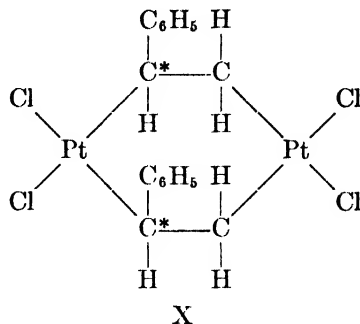
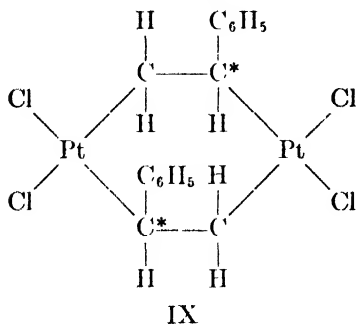
Pfeiffer (93) has proposed a formula for ethylene-platinous chloride in which platinum is tetravalent, the ethylene molecule occupies one coördination position about the platinum atom, and two of the chlorine atoms serve as bridging atoms in the dinuclear complex (formula VII). Anderson (3) accepts this formula.



Kharasch and Ashford (64) have objected to such a formula on the basis that it postulates the formation of two coördinate bonds by one chlorine atom. They have proposed a ring structure for compounds of the type $[MCl_2 \cdot Un]_2$, in which the *olefin* molecule acts as a bridging unit between the metal atoms:



With such a structure there are interesting possibilities of structural isomerism. As pointed out by Kharasch and Ashford, the compound derived from styrene, for instance, might have either of the two structures shown by the formulas IX and X:



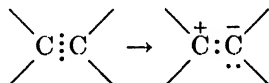
Each of these structural isomers contains two asymmetric carbon atoms and can exist in three stereoisomeric forms. The number of isomers would become still greater, of course, with more highly substituted ethylenes.

Kharasch and Ashford's objection to Pfeiffer's formula need not be given too much weight, since stable compounds are known in which halogen atoms act as bridging groups. A number of examples of such compounds have been described by Mann and Purdie (76), Gibson and Simonsen (29), Palmer and Elliott (88), and Wells (122).

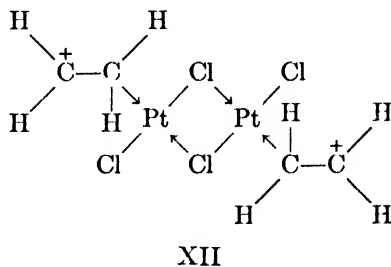
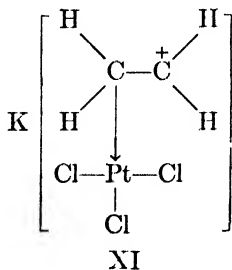
An objection may be raised to Kharasch and Ashford's formula, in that platinum does not appear to be truly in the platinous state. This might seem difficult to reconcile with their statement that it is well established that these compounds are derivatives of platinous platinum. It seems more difficult, however, to understand the mutual ease with which one olefin can replace another if the structure is of the type shown in formula VIII. In the opinion of Anderson (3), any formulation involving quadrivalent platinum introduces covalences linking the hydrocarbon to the platinum atom, and the resulting complex must necessarily be represented as an alkyl platinum derivative. Winstein and Lucas (123) feel that from a purely chemical point of view one would hardly expect these compounds to undergo so many reactions typical of compounds having a coordinate link if the carbon-to-metal bond is a metallo-organic type as in formula VIII.

As a matter of fact, it is perhaps pointless to attempt to label platinum in a cyclic compound such as proposed by Kharasch and Ashford as platinous or platinic. A structure of this type is unique and the usual terms pertaining to valence and linkages can not be used here appropriately.

Kharasch and Ashford's method of formulating the structures of the bimolecular compounds $[MCl_2 \cdot Un]_2$, is hardly applicable to compounds of the type $K[PtCl_2 \cdot Un]$. It seems very improbable that the latter substances are other than unimolecular, since the platinumous platinum has its normal coördination number of 4. Kharasch and Ashford (64), nevertheless, point out that there is no experimental evidence to support this contention. It is generally agreed that in the compounds $K[PtCl_2 \cdot Un]$ the olefinic molecule occupies only one position in the coördination sphere (3, 61, 92, 94). By our classical concepts, then, something must happen whereby a pair of electrons becomes available for coördinating purposes in the olefinic molecule. One possible mechanism by which such a pair of electrons may be produced in ethylene and similar compounds or in an aromatic nucleus has been proposed by Bennett and Willis (6). They suggest that a lone pair may be produced by a complete transfer of one pair of electrons from the double bond to one carbon, resulting in a polarized state of the molecule, and converting it at the same time into a potential "donor" molecule:



The structures of Zeise's salt and of Pfeiffer's and Anderson's ethylene-platinumous chloride would then presumably become as shown in formulas XI and XII.

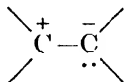


Anderson (4) critically examined the applicability of this theory to the platinum-olefin type compounds, and, while admitting that such a mechanism seems the only means whereby a lone pair of electrons can be produced at one olefinic carbon atom, indicated that it is not altogether free from objections on physical grounds. This hypothetical polarized state of the bond represents an excited state of the molecule, and it is questionable whether such an excitation would occur under the conditions used for preparing these compounds. If, on the other hand, the activation takes place in the field of the platinum atom, the reactions must still involve a

high energy of activation. If an excitation or polarization of the olefinic molecule occurs, then an abnormally low value for the net heat of formation of the coordinate link might be expected as compared with the value found for the coordination of compounds already having a free pair of electrons. In so far as the strength of a coordinate bond can be taken as a measure of its heat of formation, the linkage $\text{Pt} \leftarrow \text{olefin}$ must be associated with a smaller heat of formation than the linkage $\text{Pt} \leftarrow \text{NR}_3$. A low heat of coordination, however, is not necessarily an indication that internal rearrangement preceded coordination.

Another objection to the mechanism discussed above is that the opening of the double bond leaves one of the carbon atoms with a sextet of electrons. Such a mechanism would be expected to provide ample opportunity for polymerization and rearrangement. In fact, a reversible process in which the double bond is opened by coordination to a metal should actually promote polymerization. Anderson reports that polymerization occurred occasionally but quite irregularly. Lucas states that polymerization was not observed and that not the slightest rearrangement of olefins occurred.

If a polarized intermediate such as

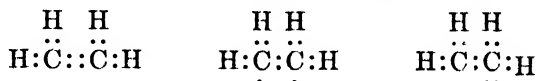


is formed in the coordination of an olefin to a metal, unsymmetrical substitution about the double bond should be a factor influencing the ability of olefins to coordinate (4). This seems to be borne out by Anderson's observation that styrene coordinates much more readily than cyclohexene. The phenyl group in styrene confers on the double bond an appreciable dipole moment (0.37×10^{-18} E.S.U.) (87). If the polar influence of this group is associated in some way with the relatively high coordinating ability of styrene, the insertion of another phenyl group on the same carbon might well lead to an even greater coordinating tendency. Anderson found the exact reverse of this to be the case. If unsymmetrical diphenylethylene formed coordination compounds with platinous chloride, they were too unstable to be isolated.

These apparent contradictions as to the rôle that dissymmetry about the double bond plays in promoting or retarding coordination have been explained by Anderson on the basis of the steric factors involved. He concludes that in the case of unsymmetrical diphenylethylene the augmented dissymmetry of the double bond may be outweighed by the steric effect of the two phenyl groups. These two bulky groups may effectively "block" any attachment to the carbon atom.

It is interesting to note in this connection that ethylene, which forms some of the most stable metal-olefin compounds, has been found to have

zero dipole moment (108). Lewis (69) supposed that the average state of ethylene is a composite of three states as indicated below:

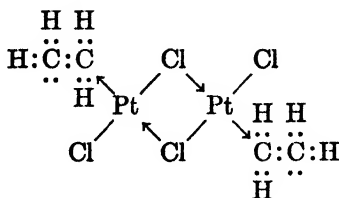


At any given instant some molecules may approximate any one of these states, but the great majority of them must be nearest to the first structure, as shown by the phenomenon of *cis trans* isomerism in olefinic molecules. Such stereoisomerism is generally ascribed to the lack of free rotation about the double bond.

The relationship, if any, between *cis*- and *trans*-configurations and coördinating ability seems somewhat obscure. Kharasch and Ashford (64) isolated crystalline compounds from *trans*-dichloroethylene and from *trans*-diphenylethylene, but could not obtain compounds from the *cis*-isomers. They did, however, obtain coördination complexes of the *cis*-compounds cyclohexene, dipentene, and pinene. Rather similar erratic results were obtained by Anderson (4), who found that both of the *cis*-compounds indene and cyclohexene coordinate, whereas *trans*-phenyl-methylethylene does not. However, the *trans*-form of pentene-2 coördinates rather strongly.

One fact which stands out and seems quite general is that progressive substitution about the double bond greatly reduces the coördinating ability of the olefins.

In attempting to overcome the necessity for assuming the existence of a trivalent carbon atom and yet retain the advantages of Pfeiffer's formula for ethylene-platinous chloride, Stiegman (112) proposed a formula (XIII) in which platinum acts as the "donor" atom and the activated or polarized olefin the "acceptor" molecule:

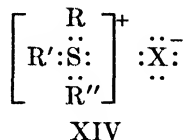


XIII

The assumption is made that in some manner the two electrons needed for the platinum-olefin bond are "called out" from the electron shells of the platinum atom. This is indicated, or implied at least, in the ring formula of Kharasch and Ashford.

, In Stiegman's formulation each carbon atom has a complete octet of

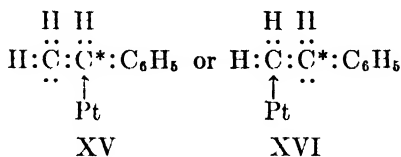
electrons, but one pair of these electrons is "unshared". Whether such a structure could have a stable existence is debatable. It is conceivable that under the influence of the pair of electrons donated from the platinum to one carbon atom the pair of unshared electrons on the other carbon atom would act much as a fourth group about this atom. This situation would be quite comparable to the case of sulfur in sulfonium salts:



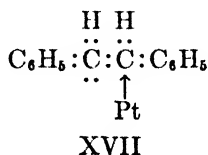
Properly substituted sulfur compounds of this type have been resolved (95, 107), indicating that the valences of the sulfur atom possess a tetrahedral distribution and that the unshared pair of electrons acts as the fourth group.

Another factor which may have some bearing on whether a structure as given above for the platinum-olefin compounds could exist is that the carbon holding the unshared pair of electrons possesses a residual negative charge.

When a properly substituted olefin is used in preparing a compound analogous to that shown in formula XIII, one or more asymmetric carbon atoms might well result. In the case of styrene platinous chloride, the platinum can donate a pair of electrons to either of the two carbon atoms:



In formula XV the starred carbon atom is asymmetric because of the presence about it of four different groups. In formula XVI the starred atom is again asymmetric if the pair of unshared electrons can function as the fourth group. In the case of stilbene only one structure is possible:

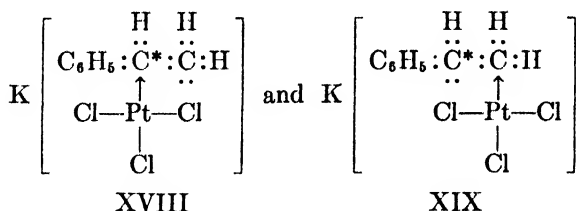


However, since these compounds are non-ionic and possess no functional groups, the usual methods of resolution are not applicable. Using the method of selective adsorption of one isomer by finely divided optically

active quartz in contact with the racemic solution (118), Stiegman (112) obtained some evidence of optical activity with the styrene- and stilbene-platinous chloride complexes. A more thorough study of the quartz method for the resolution not only of the above platinum-olefin compounds but also of compounds definitely known to be resolvable by other means casts some doubt on the reliability of this method (63). For this reason the results obtained by Stiegman must be regarded as somewhat inconclusive. Moreover, from the standpoint of the stereochemical possibilities involved, a positive resolution of these compounds by the quartz method would not distinguish between the formula suggested by Stiegman (formula XIII) and the ring structure proposed by Kharasch and Ashford (formula VIII).

An analog of Zeise's salt has been studied with a view to determining whether the ion $[\text{PtCl}_3 \cdot \text{Un}]^-$ can be resolved when a properly substituted olefin is present in the coordination sphere (63). The styrene complex was used because it appeared to be the most stable of the complexes which satisfy the stereochemical requirements of the problem. Since it is reasonably certain that the ion $[\text{PtCl}_3 \cdot \text{Un}]^-$ is monomolecular (see, however, page 251), it was thought that the ambiguities associated with compounds of the type $[\text{PtCl}_2 \cdot \text{Un}]_2$ and the rather uncertain method of resolution would be eliminated.

If the platinum acts as a "donor" atom, the styrene analog of Zeise's salt can be formulated in two ways (formulas XVIII and XIX):



In either event an asymmetric carbon atom is present (starred), and the anion exists in enantiomorphic forms. Since the compound is ionic, the usual methods of resolution can be employed. While the preliminary attempts to resolve the above compound have been unsuccessful, they are not to be taken as conclusive, and the above formulation cannot be definitely discarded.

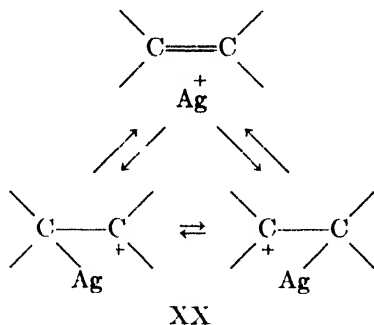
Hel'man (40) has proposed a unique explanation of the bond formation between an olefin and platinum. By virtue of the high *trans* influence (15, 38) of this group of compounds, the platinum first functions as an electron donor to the activated olefin, and then becomes an acceptor. This, according to Hel'man, results in the formation of a four-electron

covalent bond and a high activity of the inner sphere. Such a mechanism is difficult to comprehend.

Little insight into the structure of these compounds has been obtained from absorption spectra data. Anderson (4) examined the absorption spectra of the styrene and indene salts $K[PtCl_3 \cdot Un]$ over the range 2800–4300 Å. on a reflection grating spectrograph and found them to conform closely to those of other platinous complexes. Apparently no study of the infrared absorption spectra of these compounds has been made.

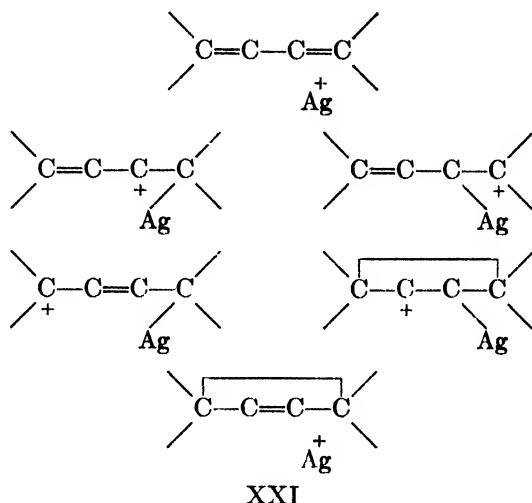
Winstein and Lucas (123), using their distribution method, have concluded that complex formation with silver ions in solution is a general property of ethylenic compounds. They state that the silver-olefin coordinate bond is probably similar in character to the coordinate bonds of other metals with olefins. Their observations indicate that complex formation is rapid and reversible. They found no rearrangement of isomeric forms of substituted olefins during their work.

From a consideration of their results, Winstein and Lucas propose a structure for these metal-olefin complexes which involves equilibria among three forms:



They call attention to the fact that a structure made up of contributions from these three forms need not result from an intermediate containing an activated double bond. Resonance among the three forms prevents the complex from behaving as a molecule having a carbon with just a sextet of electrons. Thus the facts of rapid reversible reactions but no polymerization or rearrangement are adequately explained, and the characteristic properties of these complexes seem reasonable. Also, they maintain, any objection to the formation of what amounts to a three-membered ring on the basis of strain involved is not serious, since the carbon-carbon-metal bond angle will be considerably greater than the 60° angle of cyclopropane, and the resonance energy is enough greater than the strain energy that a structure of moderate stability is to be expected.

On the basis of the same mechanism, Winstein and Lucas consider the following forms as contributing to the structures of the diene-monosilver complexes:



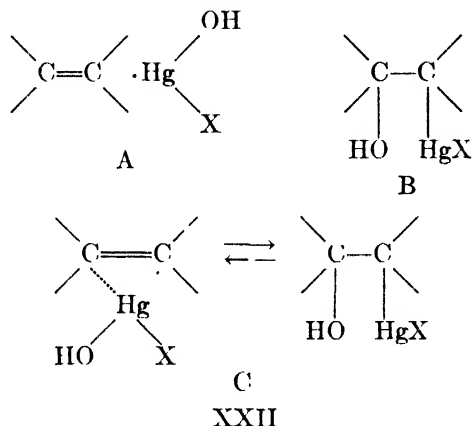
By extending the concept of resonance to the Zeise series of salts, $K[PtCl_3 \cdot Un]$, three electronic forms corresponding to those on page 256 (XX) are obtained. In the case of complexes of the type $[PtCl_2 \cdot Un]_2$, the possibility of resonance among nine electronic forms is a factor which, according to Winstein and Lucas, enhances the stability of these compounds.

Winstein and Lucas agree with Anderson that the influence of the structure of the olefinic molecule upon the stability of the resulting complexes is steric. Observations made during their study of silver olefin complexes indicate that the stability of the complex is less, the more deeply the double bond is buried in the carbon chain.

It is interesting at this point to recall Anderson's comments (3) with regard to the reaction of Zeise's salt with water (page 243). He states that the products of decomposition of this salt by water might be expected to contain either (a) ethylene glycol (or possibly ethylene chlorohydrin) if the ethylene double linkage were in some manner symmetrically coördinated (34, 91, 92) to the platinum atom, or (b) acetaldehyde if the coördinate link is formed by one of the carbon atoms. The reaction indicates that acetaldehyde is formed. Perhaps more helpful information could be obtained from a study of the reaction between water and a Zeise-type salt containing an unsymmetrical olefin such as propylene.

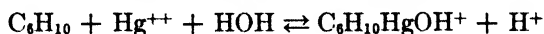
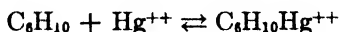
Of the mercury-olefin compounds, those with ethylene have been most

investigated. A lively controversy has centered about the structure of these and substituted ethylene derivatives. Mainly because of the ease with which the olefin can be regenerated from its combination with a mercuric salt, Manchot (73) regarded these substances as double salts or "molecular addition compounds" represented by structure A (XXII). The more common theory is that they are "true addition compounds" of type B. Sand (103), on the other hand, preferred to consider them as representing an equilibrium mixture between a molecular addition compound and the ordinary type of addition compound. He illustrated these



tautomeric forms as in C. Adams, Roman, and Sperry (1) have discussed the relative merits of these three structures and indicate their preference for type B. This structure is generally accepted at the present time and is rather conclusively supported by the fact that optically active mercury compounds with olefins of the type $RR'C=CRR'$ have been prepared (105). There are indications (104, 124), however, that in addition to products of type B, molecular or coordination compounds of olefins with mercuric salts of type A may also exist.

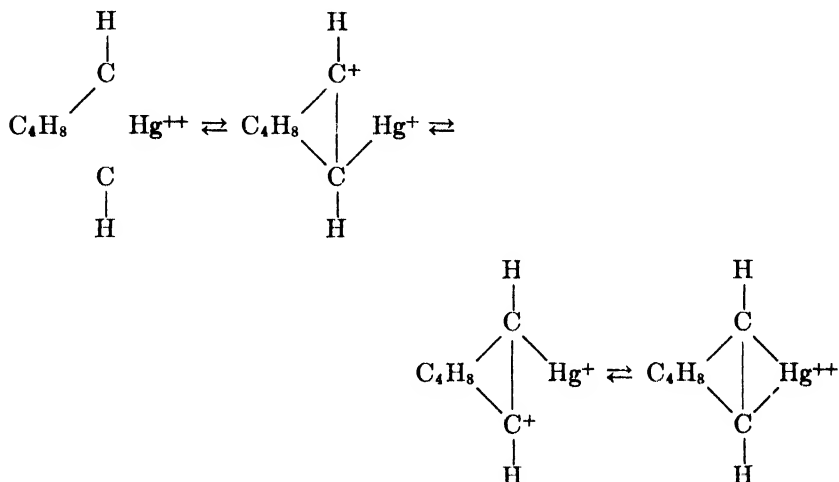
Using the distribution method (123), Lucas, Hepner, and Winstein (71) found that the mercuric ion forms rather stable complexes with cyclohexene. This hydrocarbon dissolves readily in aqueous mercuric nitrate, forming an acid solution. Two reactions which seem significant in the formation of the mercury-cyclohexene complex in solution are:



The first reaction is probably strictly analogous to the reaction of silver ion and an olefin. However, in the case of mercuric ion and cyclohexene

the reaction proceeds principally according to the second equation. Experimental data point to the formation of the 1:1 complexes $C_6H_{10}Hg^{++}$ and $C_6H_{10}HgOH^+$.

The conclusion was reached (123) that coördination complexes of mercuric ion with cyclohexene can be formulated in much the same way as the analogous silver-olefin complexes. The various resonating forms of the "cyclohexene-mercurinium" ion can be represented in simplified form as:



XXIII

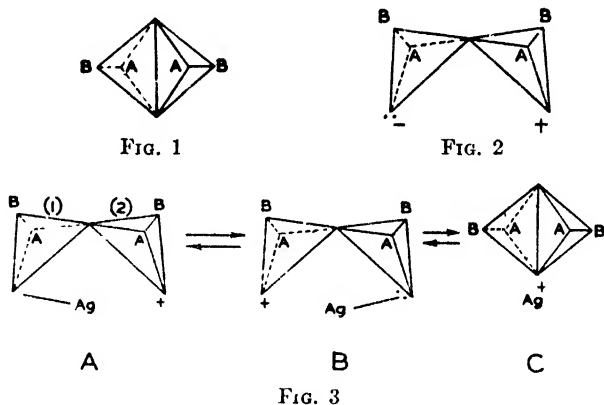
The "cyclohexene-mercurinium hydroxide" ion, $C_6H_{10}HgOH^+$, can be represented in a similar way, the mercury atom holding one hydroxyl radical. During the course of the above investigation there were indications that secondary reactions, not well understood, were occurring. One of these reactions presumably led to the formation of a compound of type B (XXII).

Lucas and coworkers admit that the discovery of coördination complexes of an olefin and mercuric ion adds confusion to our knowledge of this type of compound. The equilibria between the olefin, the mercuric salt, the compound of type A (XXII), and the compound of type B (XXII), are apparently complex, and it is entirely possible that some of the products which have been obtained in the past were actually of the coördination type.

The steps through which resonating systems pass, according to the theory advanced by Winstein and Lucas, can be pictured with the aid of three-dimensional diagrams. Figure 1 represents the *trans*-form of a substituted

olefin. The opening of the double bond can be considered as giving rise to a molecule in which one carbon atom possesses a sextet of electrons, as shown in figure 2. This, in effect, is a representation in model form of the "polarized" or "excited" state previously mentioned. However, Winstein and Lucas prefer to consider that this is *not* an intermediate in their theory of the formation of olefin-metal bonds. The resonating forms which they postulate may be represented by the diagrams shown in figure 3.

What is actually being said, apparently, when Winstein and Lucas state that there is no rearrangement during the above resonance process is that the pair of electrons indicated can in some manner "resonate" or shift position (figure 3, A) from the apex of carbon atom 1, for example, to the "unoccupied" apex of carbon atom 2 and back again before group A or group B can move to the position on carbon atom 1 which is left unoccupied



by the resonating pair of electrons. If group A or group B does shift position, isomerization or rearrangement takes place. This, however, appears not to occur. For this reason Winstein and Lucas believe that the structure shown in figure 2 should not be postulated as an intermediate, because if this polarized form had more than a fleeting existence, rearrangement would certainly be expected.

It is interesting to speculate on the stereochemical possibilities of the above structures. At the instant the carbon-silver bond is in the position pictured in A of figure 3, carbon atom 1 holds four different groups and is consequently asymmetric. The same applies to the second carbon atom in figure 3, B. With a properly substituted olefin, carbon atom 1 would never be identical with carbon atom 2, and optical activity for the whole complex should result. However, such optical activity seems very unlikely when all of the forms in such a resonating system are more closely

examined. Considering a platinum-olefin complex in which four unlike groups are situated about the double bond, it is seen from figure 4 that a total of five forms is possible:

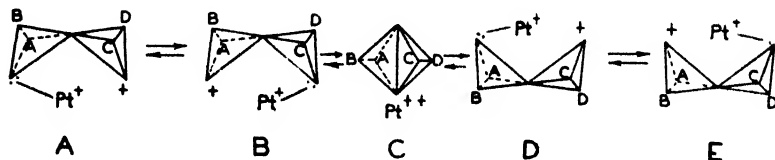


FIG. 4

When the double bond of figure 4 C "opens up," it can do so in either of two ways; in one case structures A and B (figure 4) are formed, while in the other case structures D and E (figure 4) result. However, structure A is a mirror image of structure D, and structure B is a mirror image of structure E. If the dextro- and levo-isomers of each pair of enantiomorphs are present in equal amounts, the complex is, of course, optically inactive.

It is a matter of conjecture as to whether properly chosen groups about the double bond could exert some sort of steric or polar effect and thus shift the above equilibria predominantly one way, or, in other words, cause the double bond to "open up" in one way preferentially. In this event optical activity might result. If such a situation is possible, it certainly must involve much more complex molecules than any of those discussed here.

VII. CONCLUSION

Perhaps no one theory has been advanced which satisfactorily explains all of the questions relating to the various inorganic salt-olefin compounds. It is entirely possible that the metal-olefin bonds in these complexes are not all of the same type; in that case any one theory concerning the mechanism of their formation would obviously be inadequate. The character of the metal ion involved must undoubtedly play some part in the nature and stability of the bond formed; yet it would be comforting to feel that the double bond in olefinic substances is not versatile in the manner in which it coördinates with metal ions.

Of the mechanisms proposed to explain the mode of attachment of an olefin to a metal ion, that of Winstein and Lucas seems to be of most general application. However, if cases of optical activity among these compounds are positively demonstrated, even this explanation is inadequate. A question which can logically be raised concerning this mechanism is whether or not, with the resonating type of bond suggested, the olefinic molecule truly occupies the equivalent of *only one* coördination position in the coördination sphere. Perhaps the real point of difficulty here is

lack of a clear-cut understanding of what is meant by a "coördination position". If this term implies an area or region about the central atom in which the valence bond has a certain tolerance of direction, then the above criticism may not be warranted.

The structure proposed by Winstein and Lucas resembles in some respects that suggested by Hantzsch (compare XXIII and formula III), the latter probably being equivalent, in modern terms, to a structure having single-electron bonds. It is clearly questionable, however, whether the conditions for the formation of single-electron bonds obtain in these compounds (89). The nature of the bonds in Hantzsch's formula can be explained even less satisfactorily on the basis of covalence. Either alternative—two single-electron bonds or two covalent bonds—is difficult to reconcile with the apparently well-established fact that the olefinic molecule occupies only one position in the coordination sphere.

The mechanisms proposed by Stiegman and by Kharasch and Ashford demand that a pair of electrons for the coördinate bond be supplied in some manner by the platinum. Hel'man's theory carries somewhat the same requirement. In discussing the applicability of this theory, Hel'man points out that complex compounds containing unsaturated molecules are formed only by bivalent platinum and generally by metals in their lowest state of oxidation. This would seem to imply that in some way the bivalent condition of platinum is associated with the availability of those electrons needed in forming the bond between the platinum and olefin. Since the difference in valence between platinous and platinic platinum is 2, it is conceivable that a "calling out" of two electrons, as demanded by the above theories, might be possible. However, it is exceedingly difficult to understand how any such thing could occur in the cases of Ag^+ , Fe^{++} , Fe^{+++} , Al^{+++} , Cu^+ , and Hg^{++} ions. No such assumption is necessary in applying Winstein and Lucas' theory of resonating structures.

The theory involving a polarized intermediate molecule would hardly explain the general absence of polymerization and rearrangement of the olefins. Such an intermediate might be expected to *promote* these changes. This identical theory, as a matter of fact, was used by Hunter and Yohe (51) in explaining the catalytic activity of aluminum chloride in polymerization reactions. They assumed that an intermediate "activated" complex might be formed in which one carbon atom is momentarily three-covalent. On the other hand, Winstein and Lucas' theory might appear incapable of explaining this tendency of aluminum chloride to cause polymerization. However, one must remember in this connection that these polymerization reactions are usually carried out at somewhat elevated temperatures. As previously noted, Gangloff and Henderson (27, 42) obtained crystalline addition products of aluminum chloride and various

olefinic substances at room temperature. Some polymerization at higher temperatures was noticed by Stiegman while preparing platinum-olefin compounds, and Anderson has reported occasional instances of polymerization.

It is altogether possible that at higher temperatures the resonating type of bond shifts over to an activated complex type, or the olefin receives enough energy so that it may be expelled as an activated or polarized molecule which in this excited state enters into polymerization reactions. The fact that aluminum chloride tends to induce polymerization to a greater extent than platinum and other metal ions need not be due to a fundamentally different type of metal-olefin bond but may be associated rather with a difference in the character of the metals themselves. The tendency to eliminate an olefin in an activated or polarized state or the tendency to form an activated addition complex with increase of temperature may merely be more pronounced in the case of aluminum chloride.

From theoretical considerations Winstein and Lucas (123) believe that the reactivity of these complexes is greater (a) the smaller the coordinated metal atom and (b) the more electronegative the coordinated metal atom. They feel that the relatively low reactivities of the silver and platinum complexes in terms of absence of polymerization and isomerization may be due to the comparatively large sizes of these metal atoms.

The formula of Drew, Pinkard, Wardlaw, and Cox for compounds of the type $K[PtCl_3 \cdot Un]$ does not seem satisfactory in view of the ready replacement of one olefin by another. Also, it gives platinum the unusual coordination number of 3. The structure in which the unsaturated molecule occupies one coordination position in the complex through a resonating type of bond seems preferable.

The dinuclear formula suggested by Pfeiffer for compounds of the general type $[MCl_2 \cdot Un]_2$ is preferred to that advanced by Kharasch and coworkers. Here, also, the resonating type of bond is believed preferable to that suggested by Stiegman. Such a resonating bond, however, would eliminate the possibility of optical isomerism in these dimeric compounds, unless they are of the type suggested by Kharasch and Ashford.

No definite decision can be made as to whether or not all metal-olefin bonds are of exactly the same type. Nevertheless, there seems to be no evidence to indicate that the mechanism of attachment of the olefinic molecule to the metal ion is not fundamentally the same in every case. Any variation in the properties of the resulting complexes can apparently be attributed to differences in character of the metals involved.

The author wishes to acknowledge his indebtedness to Professor John C. Bailar, Jr., for suggesting a review of this subject and to express his

gratitude for the many valuable suggestions and criticisms offered by Professor Bailar during the preparation of the manuscript.

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RELATIONSHIPS BETWEEN THE STRUCTURES AND BACTERICIDAL PROPERTIES OF PHENOLS

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Received December 2, 1940

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I. INTRODUCTION

Although phenol was suggested as a bactericidal agent by Lister in 1865, a study of the behavior of other phenolic compounds was not begun until the work in Ehrlich's laboratory which was reported in 1906 (11, 12). It was found at that time that a polyhalogenated phenol or β -naphthol was highly effective in its action on *B. diphtheriae* and *Staph. aureus*, one molecule of pentabromophenol being equivalent to five hundred molecules of phenol. It was also observed that the cresols were better germicides than phenol (5, 137). The numerous investigations on the relationship of the structure of phenolic compounds to their bactericidal properties that have been described during the past fifteen years were initiated by the work of Johnson and Lane (70) on the 4-alkylresorcinols. Their results will be discussed later.

The first standardized procedure for determining the effectiveness of a bactericidal agent was due to Rideal and Walker (125), and many of the phenol coefficients reported in the literature were determined by their method. At present a modified procedure adopted by the United States

Food and Drug Administration (160) is commonly employed. The original Rideal and Walker method and its modifications compare the relative germicidal effectiveness of a given compound with that of phenol taken as unity. The results are given on a weight rather than a molecular weight basis. The figure obtained for a given compound may vary greatly with the type of organism used, so that tests with several organisms (*B. typhosus* and *Staph. aureus* are commonly employed) are desirable. The result of an experiment at 20°C., carried out according to the Rideal-Walker method, may differ considerably from a measurement at the temperature of 37°C. commonly used now. The presence of "organic matter" likewise influences the phenol coefficient in some instances. A further complication is introduced by the fact that the bacteriostatic or antiseptic action of a compound is independent of its bactericidal or germ-killing power. A substance may be an excellent preservative, preventing the growth of organisms in high dilutions, without having a strong lethal effect. The

TABLE 1
Phenol coefficients of polyhydroxy phenols

COMPOUND	PHENOL COEFFICIENT AT 37°C.	
	<i>B. typhosus</i>	<i>Staph. aureus</i>
Catechol	0.87	0.58
Resorcinol	0.4	0.4
Hydroquinone	12.	0.44
Phloroglucinol	Negligible	Negligible
Pyrogallol	Negligible	Negligible

phenol coefficient refers to the bactericidal function. Despite these limitations, the phenol coefficients are valuable in getting an estimate of the effectiveness of compounds which are soluble enough in water for their solutions to be tested.

II. POLYHYDROXY PHENOLS

It is hardly possible to compare the bactericidal properties of phenol and benzene because of the insolubility of the hydrocarbon in aqueous media, but it is of interest to note the effect of increasing the number of hydroxyl groups attached to the nucleus. Available data (75) are listed in table 1. The behavior of hydroquinone is obviously anomalous. When the experiment was conducted at 20°C., the figures obtained for this compound dropped to 1.4 for *B. typhosus* and 0.34 for *Staph. aureus*. Earlier investigators (35) reported values for *B. typhosus*, *Staph. aureus*, and *B. coli* close to unity. Hydroquinone has a high bacteriostatic action (23) against *B. pestis*, preventing growth in a dilution of 1 to 432,000, while catechol

is about one-tenth as effective. No satisfactory explanation for the behavior of hydroquinone has been advanced. Phloroglucinol does not possess germicidal properties, although it is slightly bacteriostatic (79), as is pyrogallol.

III. HALOGENATED PHENOLS

While fluorophenol differs but little in its germicidal action from phenol (59), the chloro and bromo derivatives of phenol and resorcinol are more effective than the unsubstituted compounds. Little is known about iodinated phenols; they are relatively insoluble in water and possess an unpleasant and persistent odor which decreases the possibility of their practical use. Table 2 summarizes the data of Klarmann (82) on chloro and bromo compounds. Many of these values were greatly reduced when the determinations were made in the presence of organic matter. It is difficult

TABLE 2
Phenol coefficients of halogenated phenols

COMPOUND	PHENOL COEFFICIENTS OF CHLORINE DERIVATIVES		PHENOL COEFFICIENTS OF BROMINE DERIVATIVES	
	<i>B. typhosus</i>	<i>Staph. aureus</i>	<i>B. typhosus</i>	<i>Staph. aureus</i>
2-Halophenol .	3 6	3.8	3.8	3 7
3-Halophenol .	7.4	5 8		
4-Halophenol	3.9	3 9	5.4	4 6
2,4-Dihalophenol	13	13	19	22
2,4,6-Trihalophenol	23	25		
4-Haloresorcinol	0.7	1.0	1 0	1.3
4,6-Dihaloresorcinol. .	3 2	3 9	4 0	4 5
2,4,6-Trihaloresorcinol	5 0	4.3	6.4	6.4

to compare these figures with the earlier results of Bechold and Ehrlich (12) already mentioned, who reported that tribromo- β -naphthol kills *Staph. aureus* in 2 to 3 min. in a dilution of 250,000 and that the dibromo compound was active in a dilution of 32,000 toward *B. coli*. 2,4,5-Trichlorophenol (98), and particularly pentachlorophenol (25, 64, 124), have received attention as preservatives for commercial products. Engelhardt (47) reported that *p*-chlorophenol showed germicidal effectiveness only in a solvent of high dielectric constant; this was not the case for phenol. Halogen derivatives of phenols containing other substituents will be considered later.

IV. ALKYLPHENOLS

A considerable amount of information is now available concerning the effect of alkyl groups on the bactericidal properties of mono-, di-, and tri-

hydroxyphenols, and a number of generalizations are possible. In the homologous series of *p-n*-alkylphenols the maximum bactericidal action is reached with the *n*-amyl compound (37) when *B. typhosus* is the test organism, using the Rideal-Walker procedure. The data are listed in table 3. Because of the slight solubility of the phenols in water, they were dissolved in very dilute sodium hydroxide which in itself was not bactericidal. Against *Staph. aureus* at 20°C. a much lower phenol coefficient of 52 has been reported for *p-n*-butylphenol (118, 120), while for measurements at 25°C. a value of 68 has been found (135). More recently, deter-

TABLE 3

Phenol coefficients of alkylphenols against B. typhosus at 20°C.

<i>p</i> -ALKYLPHENOL	PHENOL COEFFICIENT
Methyl	2.5
Ethyl	7.5
<i>n</i> -Propyl	20
<i>n</i> -Butyl	70
<i>n</i> -Amyl	104
<i>n</i> -Hexyl	90
<i>n</i> -Heptyl	20

TABLE 4

Phenol coefficients against Staph. aureus at 37°C.

<i>p</i> -ALKYLPHENOL	PHENOL COEFFICIENT
Ethyl	10
<i>n</i> -Propyl	14
<i>n</i> -Butyl	21
<i>n</i> -Amyl	20
<i>n</i> -Heptyl	21

minations run at 37°C., using 30 per cent alcohol as the solvent for stock solutions of the alkylphenols, have given even lower values for the phenol coefficients (105). These are listed in table 4.

Within the experimental error, the position of the alkyl group has no effect (37, 118, 120). The three cresols and the three *n*-butylphenols are practically identical in their bactericidal action. The *o*- and *p*-sec-butylphenols have a phenol coefficient of 28, and the branching of the carbon side chain, as in *tert*-butylphenol, reduces the effectiveness to about 20. However, the condensation of 2-ethylbutanol with phenol, which might be expected to give *tert*-hexylphenols, gives two products having relatively

high coefficients. One isomer (b.p. 98–100°C. at 1.5 mm.; $n_D^{27} = 1.5133$) gave 109 against *Staph. aureus* and 86 against *E. typhi*. The other isomer (b.p. 108–110°C. at 1.5 mm.; $n_D^{27} = 1.5125$) was found to give 118 and 68, respectively, as the coefficients (59a). Since these compounds appear to be exceptions to the generalization that the straight-chain primary alkylphenols are more effective than their isomers, it would be of interest to know their structures. A phenol coefficient of 125 has been found for *o*-cyclohexylphenol against *Staph. aureus* by the F. D. A. method at 37°C. (162).

The *n*-alkylphenols are in general more difficult to prepare than their isomers. The most satisfactory synthesis involves the rearrangement of an aryl ester to the ketone by the Fries method, followed by reduction of the ketone with amalgamated zinc and hydrochloric acid (37). Another general method consists in the condensation of an aldehyde with phenol and the pyrolysis of the polymeric condensation product (105). The yield of crude *p*-alkylphenol amounts to about 40 per cent by weight of the polymer. On the other hand, the secondary and tertiary alkylphenols are obtainable directly by condensing phenol with an alcohol, alkyl halide, or olefin,—reactions that have received extensive investigation in recent years, particularly for patent purposes (18, 21, 22, 61, 94, 101, 107, 110, 113, 141).

It has been claimed that the condensation of phenol with *n*-heptyl alcohol (49) gives *n*-heptylphenol, but it seems likely that the product was a mixture. *n*-Butyl alcohol (121) yields a mixture of *sec*-butyl phenols when zinc chloride is the condensing agent, while it is claimed that aluminum chloride gives the *n*-alkylphenols with both *n*-propyl and *n*-butyl alcohols (158). *o*-Isobutylphenol has been prepared by the rearrangement of methallyl phenyl ether and the reduction of the resulting methallylphenol (8).

The presence of two alkyl groups in the phenol nucleus yields compounds that are highly effective as germicides and incidentally of very low solubility in water. The six isomeric xylenols (91) do not differ greatly in bactericidal properties, the 2,5-dimethylphenol being the most active. Carvacrol and thymol (135) both have a phenol coefficient of 28 at 25°C. against *Staph. aureus*, and the variation in the activity of isomeric *n*-alkylcresols is greater than the experimental error in only a few instances. Data for these compounds (37) are given in table 5A for *B. typhosus* at 20°C.

Three isomeric methylethylphenols (105) have also been tested against *Staph. aureus* at 37°C., with the results shown in table 5B.

The high germicidal effectiveness of the *n*-amylcresols listed in table 5A has led to their extensive study and recommendation for general use (6, 36, 38, 92). The compounds obtained by condensing "amylene" (30) or

an amyl alcohol (57, 142) with a cresol have also been described as useful bactericides. Other patents have dealt with the general preparation of secondary or tertiary alkylcresols (24, 115) as compounds having germicidal

TABLE 5A

Phenol coefficients against B. typhosus at 20°C.

ALKYL GROUP	PHENOL DERIVATIVE			
	4-Alkyl-3-methyl-	2-Alkyl-4-methyl-	4-Alkyl-2-methyl-	2-Alkyl-6-methyl-
Ethyl	12 5	12.5	15	
n-Propyl	34			
n-Butyl	100	95	110	60
n-Amyl	280	250	300	250
n-Hexyl	275	175	100	180

TABLE 5B

Phenol coefficients against Staph. aureus at 37°C.

PHENOL	PHENOL COEFFICIENT
2-Methyl-4-ethylphenol	11
3-Methyl-4-ethylphenol	10
4-Methyl-2-ethylphenol	10

TABLE 6

Phenol coefficients of products formed from cresols and 2-ethyl-1-butanol

PHENOL USED	BOILING POINT OF PRODUCT AT 1.5 MM. °C.	PHENOL COEFFICIENT	
		<i>Staph aureus</i>	<i>E. typhi</i>
o-Cresol	(a) 104-106	94	43
	(b) 110-112	183	45
m-Cresol	(a) 106-108	151	54
	(b) 114-116	231	71
p-Cresol	(a) 106-108	180	47
	(b) 115-117	(Insoluble)	(Insoluble)

properties. Raiziss and Clemence (115) have suggested that the condensation of a cresol with 2-methyl-1-butanol may give the primary alkylcresol, but this is unlikely in view of the results reported in condensations involving phenol. The phenol coefficient of "sec-amyl-o-cresol" against

Staph. aureus at 37°C. is 125 and for "sec-amyl-*p*-cresol" it is 100. For a more complex mixture, "tert-amyltricrosol", the value of 62.5 was obtained (162).

The condensation of 2-ethyl-1-butanol with the three cresols gives in each instance two products. In table 6 are included the available data for these (59a).

In addition, *m*-cresol was condensed with "sec-hexanol" to give two products, boiling at 111–113°C. and at 118–120°C., which gave coefficients of 225 and 237 against *Staph. aureus*; against *E. typhi* the first product gave the coefficient 92. In a large group of methyl- and dimethyl-isobutylphenols (8), the most effective compound tested against *Staph. aureus* at 37°C. was 2-isobutyl-4,5-dimethylphenol, which had a phenol coefficient above 50. It was concluded that the isobutyl compounds were probably less effective than their *n*-butyl isomers.

V. ALKYLRESORCINOLS

Because of the interesting results obtained in the investigation by Johnson and Lane (70), the preparation and properties of alkylated resorcinols have been thoroughly studied. The primary 4-alkylresorcinols, both normal and branched chain, are readily obtained by condensing resorcinol with the proper fatty acids in the presence of zinc chloride (42, 43, 44, 70, 71), followed by reduction according to the method of Clemmensen (34). Secondary and tertiary 4-alkylresorcinols have been prepared by condensing olefins, alkyl halides, or alcohols directly with resorcinol (2, 21, 31, 106, 107, 126, 128), while the synthesis of the 5-*n*-alkylresorcinols (152) and of the 2-alkylresorcinols (130) involves a long series of reactions.

The several investigations on the bactericidal properties of the 4-alkylresorcinols illustrate the variability of the values for the phenol coefficient obtained with various strains of *B. typhosus* (42, 43, 44, 54, 77, 123, 135, 136) and with what appear to be minor variations in the test conditions. Apparently the variation is less serious in the case of tests using *Staph. aureus*. In table 7 are listed the results given by Dohme, Cox, and Miller (44) for *B. typhosus* and those of Schaffer and Tilley (135, 136) for *Staph. aureus*. Whereas the germicidal action toward *B. typhosus* reaches a maximum with the *n*-hexyl compound, the effect on *Staph. aureus* increases continuously with the length of the side chain. As in the alkylphenols, branching of the carbon chain reduces the effectiveness. Because of the bactericidal potency and the low toxicity of 4-*n*-hexylresorcinol, it has come into general use.

The 5-*n*-alkylresorcinols (152) show about the same germicidal activity against *Staph. aureus* as do the 4-isomers as high as the *n*-amyl compound, but above this their effectiveness is less (table 8). Against *B. typhosus* at

20°C., 5-*n*-hexylresorcinol has a coefficient of 22, compared with 50 for the 4-isomer. The 2-alkylresorcinols are relatively ineffective bactericidal agents (130). Although many *sec*-alkylresorcinols have been described (2, 21, 31, 106, 107, 126, 128) as having disinfectant action, phenol coefficients were not given. 4-Cyclohexylresorcinol (7) gave a value of 23 to 27, when tested by the United States Hygienic Laboratory method, which is close to that for isohexylresorcinol. Cyclohexylmethyl-, β -cyclohexyl-

TABLE 7
Phenol coefficients of 4-alkylresorcinols[†]

4-ALKYLRESORCINOL	PHENOL COEFFICIENT	
	<i>B. typhosus</i>	<i>Staph. aureus</i>
<i>n</i> -Propyl	5	3.7
<i>n</i> -Butyl	22	10
Isobutyl	15	
<i>n</i> -Amyl	33	30
Isoamyl	24	
<i>n</i> -Hexyl	46 to 56	98
Isohexyl	27	
<i>n</i> -Heptyl	30	280
<i>n</i> -Octyl	0	680
<i>n</i> -Nonyl	0	980

TABLE 8
Phenol coefficients against Staph. aureus at 37°C.

ALKYLRESORCINOL	PHENOL COEFFICIENT	
	4-Isomers	5-Isomers
<i>n</i> -Propyl	4	5
<i>n</i> -Butyl	10	10
<i>n</i> -Amyl	30	35
<i>n</i> -Hexyl	98	49
<i>n</i> -Heptyl	280	128

ethyl-, and cyclopentylmethyl-resorcinols are less effective than *n*-hexylresorcinol (155); exact figures were not given. 4-Hexenylresorcinol is an active bactericide (67); the phenol coefficient was reported to be 150 against *Staph. aureus* at 37.5°C., 200 against *Strep. hemolyticus* at 37.5°C., and 40 against *B. typhosus* at 20.5°C. 4-Pentenylresorcinol was much less active.

A few dialkylresorcinols (2, 3, 74, 140) have been tested; 4,6-diethylresorcinol is as effective as *n*-butylresorcinol, but the di-*n*-propyl compound has a phenol coefficient of only 18; di-*n*-butylresorcinol is no more

bactericidal than the *n*-butyl compound, and the di-*n*-hexyl derivative is less than one-half as effective as *n*-hexylresorcinol. What are presumably di-*sec*-alkylresorcinols have been prepared (117) by condensing alcohols with resorcinol in the presence of zinc chloride. Since there may be some question about the structures of these compounds, particularly since their boiling points are lower than would be expected, there are listed in table 9 the alcohol used in preparing each compound and also the boiling point of the dialkylresorcinol.

The phenol coefficients were determined by the United States Hygienic Laboratory method. The unusually high values shown by the *sec*-hexyl and the heptyl compounds are surprising, in view of the small activity of the lower members of the series. The solubility of these compounds is only one part in 20,000 to 40,000 parts of water, which makes proper evaluation difficult (88, 96).

TABLE 9
Properties of "di-*sec*-alkylresorcinols"

ALCOHOL	BOILING POINT OF PRODUCT °C.	PHENOL COEFFICIENT	
		<i>Staph. aureus</i>	<i>Strep. hemolyticus</i>
C ₂ H ₅ OH	135-137 (5 mm.)	60	65
<i>n</i> -C ₃ H ₇ OH	156-158 (7 mm.)	20	25
<i>n</i> -C ₄ H ₁₁ OH	168-175 (7 mm.)		
<i>sec</i> -C ₄ H ₁₁ OH	119-122 (1 mm.)	190	235
<i>n</i> -C ₆ H ₁₃ OH	178-182 (7 mm.)	1000	1350
<i>n</i> -C ₇ H ₁₅ OH	165-175 (2 mm.)	525	525

VI. ALKYL CATECHOLS AND ALKYLHYDROQUINONES

Comparatively recently, several 4-*n*-alkylcatechols have been prepared by both the Clemmensen reduction and catalytic reduction of the corresponding ketones, and the phenol coefficients of three members of the series determined against *Staph. aureus*. These are appreciably higher for the *n*-butyl (29 versus 10) and *n*-hexyl (129 versus 98) compounds, but lower for the *n*-heptyl (177 versus 280) than in the case of the corresponding resorcinol derivatives. Di-*sec*-hexylhydroquinone (117) gave a value of 25 against *Staph. aureus* and 38 against *Strep. hemolyticus*.

VII. ALKYLPHLOROGLUCINOLS AND ALKYL PYROGALLOLS

Several alkyl derivatives of trihydroxyphenols have been investigated as bactericidal agents. The *n*-hexylphloroglucinol (74) has a phenol coefficient of 8, while that for the triethyl compound is 2.5. What is prob-

ably a di-*sec*-hexylphloroglucinol made by condensing *n*-hexyl alcohol with phloroglucinol in the presence of zinc chloride (117) was found to be about ten times as effective against *Strep. hemolyticus* as against *Staph. aureus* (125 versus 12). The 4-*n*-alkylpyrogallol series shows a maximum effect

TABLE 10

Phenol coefficients of derivatives of pyrogallol, determined by the F.D.A. method at 37.5°C.

PYROGALLOL DERIVATIVE	PHENOL COEFFICIENT	
	<i>Staph. aureus</i>	<i>B. coli</i>
Ethyl	1 0	2.3
<i>n</i> -Propyl	2.5	4 4
<i>n</i> -Butyl	5 0	12.6
<i>n</i> -Amyl	19 0	25
<i>n</i> -Hexyl	44	38
<i>n</i> -Heptyl	50.	26
Heptenyl	120	
Dihexenyl I	20	<11 *
Dihexenyl II	250	<11. *
Diheptenyl	20	

* Against *B. typhosus* at 20.5°C.

TABLE 11

Phenol coefficients of di-sec- and di-tert-alkylpyrogallols

ALCOHOL USED	BOILING POINT OF PRODUCT °C.	PHENOL COEFFICIENT	
		<i>Staph. aureus</i>	<i>Strep. hemolyticus</i>
<i>n</i> -Butyl	136-140 (2 mm.)	90	100
<i>tert</i> -Butyl	165-170 (4 mm.)	5	3
<i>n</i> -Amyl	146-148 (1.5 mm.)	200	220
<i>tert</i> -Amyl	150-153 (2 mm.)	100	100
Isoamyl	157-159 (2 mm.)	11	15
1-Methylbutyl..	157-160 (2 mm.)	215	235
1-Ethylpropyl	154-158 (2 mm.)	118	190
2-Methylbutyl	146-148 (2 mm.)	95	145
<i>n</i> -Hexyl	153-155 (1.5 mm.)	280	320
<i>n</i> -Heptyl	160-164 (1.5 mm.)	360	375
<i>n</i> -Octyl	168-172 (1.5 mm.)	235	

against *B. coli* at the *n*-hexyl compound, while with *Staph. aureus* the effect is still rising with the *n*-heptyl derivative (58). Included in table 10 are figures for some alkenylpyrogallols (66) having high potency against *Staph. aureus* and *Strep. hemolyticus*. Phenol coefficients have been reported for

a number of products believed to be di-*sec*- and di-*tert*-alkylpyrogallols, made by condensing alcohols with pyrogallol in the presence of zinc chloride (117). The properties of these compounds are listed in table 11. The most striking feature of the results is the wide variation in the effectiveness of the isomeric amyl compounds. The products obtained from *n*-amyl and the two *sec*-amyl alcohols are probably all mixtures of di-*sec*-amylpyrogallols, and the phenol coefficients are in accord with this. The product obtained from isoamyl alcohol should be the *tert*-amyl derivative, but it had quite different bactericidal properties from those of the compound made from *tert*-amyl alcohol. Other inconsistencies in the results indicate the necessity for information regarding structures before conclusions can be drawn.

TABLE 12
Properties of 2-alkyl-4-fluorophenols

ALKYL GROUP	SOLUBILITY IN WATER	PHENOL COEFFICIENT	
		<i>B. typhosus</i> at 20°C.	<i>Staph. aureus</i> at 37°C.
	<i>grams per liter</i>		
Ethyl	3.51	10	
<i>n</i> -Propyl	1.95	21	
<i>n</i> -Butyl	0.76*	66	60
<i>n</i> -Amyl	0.27†	69	139
<i>n</i> -Hexyl	0.18*	<62	

* In 20 per cent ethyl alcohol.

† 0.41 in 20 per cent ethyl alcohol.

VIII. ALKYLHALOPHENOLS AND ALKYLHALORESORCINOIS

Since alkyl groups and halogen atoms separately increase the bactericidal activity of a phenol, it is interesting to note the cumulative effect when both types of substituents are present. Alkylhalophenols have been prepared by methods analogous to those used in making the unhalogenated compounds.

The presence of fluorine para to the hydroxyl group has a smaller effect on the germicidal properties of alkylphenols (145) than is obtained with the other halogens. The data are given in table 12. A *p*-fluoro-*o*-pentenylphenol has also been prepared (40); it was claimed that it has germicidal activity.

Klarman and coworkers (81, 84, 86) have reported the behavior of six varieties of microorganisms toward numerous alkylchlorophenols. In table 13 are listed the results obtained with *o*-alkyl-*p*-chlorophenols. In-

spection of these data shows that, as in previous groups of compounds, the first three members of the series have about the same activity toward all microorganisms, but with increasing length of side chain a maximum value is reached for each organism. Since this maximum occurs with different lengths of side chains, certain compounds are highly effective toward some organisms and all but inert to others. Klarmann has used the term "quasi-specific" activity to designate this phenomenon. It has been found among the higher members of all series of alkylated phenols wherever the bactericidal properties have been studied for several organisms.

The bactericidal action of *o*-chloro-*p*-alkylphenols is on the whole less than for the series just described. The data are presented in table 14.

TABLE 13
Phenol coefficients of o-alkyl-p-chlorophenols

ALKYL GROUP	PHENOL COEFFICIENT					
	<i>E. typhi</i>	<i>E. paradyenterias</i>	<i>Staph. aureus</i>	<i>Strep. hemolyticus</i>	<i>Mycobacterium smegmatis</i>	<i>Trichophyton roseaceum</i>
None	4 3	4.7	4 3	4 4	3 9	4.2
Methyl	12 5	14 3	12 5	11.1	13.3	11.7
Ethyl	28 6	32 1	34 4	31 3	25	27.5
<i>n</i> -Propyl	93.	100	94	78.	89	83
<i>n</i> -Butyl.	141	167.	257	250	156	160.
<i>n</i> -Amyl	156	200	500	556	400.	400
<i>n</i> -Hexyl	(23 2)	333.	1250.	1333.	1111.	500.
<i>n</i> -Heptyl		133	1500.	2220.	1250.	667.
<i>n</i> -Octyl		(26 7)	1750.	>667.	156.	
<i>sec</i> -Amyl	46 7	80.	312.	312.	389.	250.
Cyclohexyl		80.	438.	361.	278.	300.
<i>sec</i> -Octyl.			1000.	>555.	>100.	>50.

Here the quasi-specific behavior does not become important until there are more than four carbon atoms in the side chain. It may be that an interaction of the hydroxyl and chloro groups is responsible for the decreased effectiveness of these compounds as compared with their isomers.

Results similar to those shown in table 14 were reported the same year by Blucke and Stockhaus (13). These investigators mentioned the erratic behavior of the hexyl and heptyl derivatives, the phenol coefficients using *Staph. aureus* varying from 444 to 714 and from 375 to 666 for the two compounds.

3-Methyl-4-chlorophenol is slightly less effective than the *o*-cresol derivative (81). Rapps (116) found this compound to be about twice as effective in a castor oil soap solution as in water, the Rideal-Walker phenol coeffi-

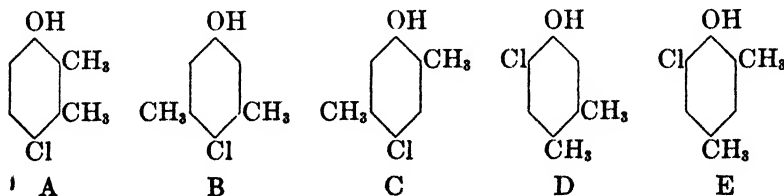
cient rising from 13 to 25. Chlorine derivatives of other 3-alkylphenols are not known, chiefly because of the large amount of work that would be involved in preparing them.

A mixture of chloro-*sec*-butylphenols obtained by the action of sulfuryl chloride upon *sec*-butylphenol has been patented for its bactericidal activity (14). The dichlorination of the cresols has been reported to multiply their germicidal effectiveness by ten (156). Commercial chloro-*o*-cyclohexylphenol gives a phenol coefficient of 437 against *Staph. aureus* (162). A series of 2,4-dichloro-6-alkylphenols has recently been prepared (26), but unfortunately there seems to be no information available concerning their bactericidal power. The preparation of a pentenyl-*m*-chlorophenol has also been described (40) and compounds of this type were claimed as bactericides.

TABLE 14
Phenol coefficients of p-alkyl-o-chlorophenols

ALKYL GROUP	PHENOL COEFFICIENT					
	<i>E. typhi</i>	<i>E. paratyphosae</i>	<i>Staph. aureus</i>	<i>Strep. hemolyticus</i>	<i>Mycobacterium smegmatis</i>	<i>Trichophyton rosaceum</i>
None	2 5	2 3	2.9	2.0	2.2	<1
Methyl	6 3	5 3	7.5	5.6	6.3	7.0
Ethyl	17 2	13 3	15 7	15.0	15.6	14.0
<i>n</i> -Propyl	38	40	32	35	33	>33
<i>n</i> -Butyl	87	80	94	89	125	80
<i>n</i> -Amyl	80	80	286	222	250	>250
<i>tert</i> -Amyl	(32)	47	125	138	138	145
<i>n</i> -Hexyl		(36)	714	625	500	>420
<i>n</i> -Octyl			375	350	200	>290

A study of the isomeric chloroxylenols has shown that the compounds A, B, and C were about four times as effective against *B. coli* and fifteen times as strong against *Staph. aureus* (60, 90, 91) as the compounds D and E. Here again, the less active substances are those having chlorine ortho to hydroxyl. For B the phenol coefficient by the Rideal-Walker procedure is 38 (116).



Klarmann and coworkers have studied an interesting group of di- and trialkyl-*p*-chlorophenols. The germicidal activity of these compounds is tabulated in table 15. One trialkyl-2-chlorophenol,—namely, the 4-*n*-propyl-3,5-dimethyl compound,—was also studied. The figures obtained were 75, 2000, 140, 222, and 100 for the five microorganisms listed in table 15. It is obvious that when the number of carbon atoms in the side chain totals more than four, the bactericidal effect toward *E. typhi* drops below the maximum value, whereas with the other organisms the maximum is not reached until the carbon atoms total seven. As Klarmann points out, a compound having the side-chain carbon atoms in one alkyl group is

TABLE 15
Phenol coefficients of di- and tri-alkyl-4-chlorophenols

ALKYL GROUPS	PHENOL COEFFICIENT				
	<i>E. typhi</i>	<i>Staph. aureus</i>	<i>Strep. hemolyticus</i>	<i>Mycobacterium smegmatis</i>	<i>Trichophyton rosaceum</i>
3,5-Dimethyl	30	26	28	28	25
3-Methyl-6-ethyl	64	50	56	56	60
3-Methyl-6- <i>n</i> -propyl	133	200	178	156	150
3-Methyl-6-isopropyl (chlorothymol)	107	150	138	138	140
3-Methyl-6- <i>sec</i> -butyl	43	344	333	361	275
3-Methyl-6- <i>sec</i> -amyl	27	688	556	625	500
3-Methyl-6- <i>sec</i> -octyl		>89	122	>70	
3,5-Dimethyl-2-ethyl	46	106	94	122	130
3,5-Dimethyl-2-isopropyl	81	313	313	325	275
3,5-Dimethyl-2- <i>sec</i> -butyl	29	563	556	556	545
3,5-Dimethyl-2- <i>sec</i> -amyl*		750	1111	700	
3,5-Dimethyl-2-diethylcarbinyl*		1143	1000	667	700
3,5-Dimethyl-2- <i>sec</i> -octyl		100	>67		

* These two phenols are probably mixtures of the two straight-chain *sec*-amyl compounds in varying proportions.

more potent than when these are scattered among two or more substituents. However, because carvacrol and thymol are readily available, chlorine derivatives of these have been advocated for use as germicidal agents (111, 112, 129). Chlorine derivatives of the amylcresols have also been described in the patent literature (114).

The behavior of alkylbromophenols (80, 85) parallels that of the chlorine compounds. Available data for derivatives of *p*-bromophenol are listed in table 16. A comparison with table 13 shows that the maximum effect against *E. typhi* is reached one carbon atom lower in the series but that it is the same maximum. Against *Staph. aureus* the *n*-hexyl chloro and

bromo compounds have identical effects. Because of the lower molecular weights of the chlorine compounds, identical phenol coefficients mean a greater efficiency per molecule for the bromine compounds. The activity of 4-*n*-hexyl-2-bromophenol is just one-half that of the isomer with the bromine in the para-position and slightly less than for the chlorine compound. A series of 2,4-dibromo-6-alkylphenols has been prepared (27), but their germicidal properties were not reported.

TABLE 16
Phenol coefficients of o-alkyl-p-bromophenols

ALKYL GROUP	PHENOL COEFFICIENT			
	<i>E. typh</i>	<i>Staph. aureus</i>	<i>Mycobacterium tuberculosis</i>	<i>Monilia albicans</i>
None	6	5	5 6	6.3
Methyl	12.5	11 3	13.3	13.3
Ethyl	31.	25	28.	28.
<i>n</i> -Propyl	63.	63.	78	78.
<i>n</i> -Butyl	156.	313.	278.	222.
<i>n</i> -Amyl	63.	571.	444.	278.
<i>n</i> -Hexyl		1250.	778.	278.
<i>sec</i> -Amyl	> 33	150	156.	150.
Cyclohexyl	> 23	429.	278.	222.

TABLE 17
Phenol coefficients of 4-alkyl-6-chlororesorcinols

ALKYL GROUP	PHENOL COEFFICIENT	
	<i>Staph. aureus</i>	<i>B. typhoeus</i>
None	1.34	1 20
Ethyl	6	
<i>n</i> -Butyl	35-45	47.
<i>n</i> -Hexyl	240.	
<i>n</i> -Heptyl	625.	
<i>n</i> -Octyl	665.	

The isomeric bromoxylenols differ even more than the chlorine derivatives in their bactericidal properties. Those with the bromine atom para to the hydroxyl group are six times as strong against *B. coli* and fifteen times as active toward *Staph. aureus* as the *o*-bromo compounds. The more active compounds had phenol coefficients in the range 50 to 60.

Iodinated alkylphenols have been little studied. Their solubility in water is low (139). It has been claimed that an iodinated carvacrol (111) has useful germicidal properties.

Some information is available concerning the alkylchlororesorcinols. The presence of the chlorine practically doubles the bactericidal action, as compared to the chlorine-free compounds (99, 103, 119). A comparison of tables 7 and 17 brings out this point. Which member of the series gives the maximum effect against *B. typhosus* apparently has not been determined.

Methods for preparing a number of secondary and tertiary alkylchlororesorcinols have been described (1, 4, 102), but details of the bactericidal activity of these compounds are lacking. In one patent bromine and iodine derivatives are referred to also (87).

IX. HYDROXYCARBOXYLIC ACIDS AND THEIR DERIVATIVES ✓

Many investigations have been made on the antiseptic or preservative effect of the hydroxy acids, their halogen derivatives, and particularly their esters. Most of the results are not easily comparable with phenol coefficient values, as they deal with the bacteriostatic properties of these compounds and are frequently referred to yeast rather than to pathogenic microorganisms. However, the effects of halogen and alkyl groups are comparable to those already described for the simpler phenols and will be discussed briefly.

5-Fluorosalicyllic acid has been prepared (154) and was found to have a higher toxicity to white mice than salicylic acid but no bactericidal data were obtained. In cultures of *E. typhi*, chloro- and dichloro-salicylic acids had about the same effect as salicylic acid (127), but 5-bromosalicylic acid was two times and 3,5-dibromosalicylic acid eight times as toxic to the organism. Against *Staph. aureus* both chlorine compounds were about twice as effective as salicylic acid, while 5-bromosalicylic acid was eight times and 3,5-dibromosalicylic acid was sixty-four times as effective. A more recent study (41), using the sodium salts of the acids, showed the superiority of the halogenated salicylic acids over phenol as a growth-arresting or bacteriostatic agent, but salicylic acid and its chlorine derivatives were found to be less germicidal than phenol, while the bromine and iodine derivatives are more active.

A series of *n*-alkylsalicylic acids (39) has been prepared; it was reported that these had higher phenol coefficients than salicylic acid, but no numerical values were given. The synthesis of *sec*- and *tert*-alkylsalicylic acid derivatives has been outlined in a patent (19). The products were claimed to have disinfectant properties.

While esters of salicylic acid have little germicidal or bacteriostatic action, certain alkyl *p*-hydroxybenzoates are highly effective, particularly as preservatives (10, 46, 48, 131, 132, 144, 159). The presence of halogen (88a, 138) in the nucleus increases this effectiveness as does the nitro group

(133) also, while the amino group decreases it. Nuclear-alkylated esters of *p*-hydroxybenzoic acid have been described (53). The action of numerous dihydroxybenzoic acids and their esters on yeast has also been mentioned. A recent determination of the phenol coefficients for several *n*-alkyl 3,5-dihydroxybenzoates (153) against *Staph. aureus* at 37°C. indicated that the figures for the ethyl and *n*-butyl compounds were less than 10, while the *n*-heptyl compound gave a value of 38. .

X. NITRO-, AMINO-, AND SULFO-PHENOLS

Not a great deal is known concerning the germicidal properties of nitrophenols. Mazetti (93) found that of the three isomers the para-compound was most active. The ortho- and meta-isomers behaved about the same toward *E. typhi*, whereas against *Staph. aureus* the ortho-isomer was more effective. All three compounds were stronger bactericides than phenol. Since the pH of the nitrophenol solutions was about the same (93), their variation was not due to differences in their acid properties. *o*-Aminophenol and various of its *n*-alkyl derivatives have been described in the patent literature as highly active germicidal agents of low toxicity (109) but have not been studied extensively.

A mixture of phenolsulfonic acids of rather doubtful germicidal value, which contained chiefly the para-isomer (51, 108), was on the market for many years. In a study of sodium *p*-alkylphenolsulfonates (151) from *n*-propyl to *n*-hexyl, it was found that only the *n*-propyl and *n*-butyl compounds had measurable phenol coefficients, the values being 1.8 and 2.4, respectively. At 26°C. the solubility of the four sulfonates in water ranged from 1.5 to 0.5 g. per 100 ml. The solutions foamed readily, indicating a low surface tension. More recently, *n*-hexylresorcinolsulfonic acid has been patented (89) as a germicidal agent.

XI. HYDROXYARYL ALKYL ETHERS AND SULFIDES

Monoalkyl ethers of dihydroxyphenols may be looked upon as alkylphenols in which an oxygen has been introduced between the alkyl group and the ring, or alternatively as alkylphenols in which a methylene has been replaced by oxygen. In table 18 are listed the phenol coefficients for some monoalkyl ethers of the isomeric dihydroxy phenols (75, 77). It is obvious that the effect of the oxygen is complex. While isomeric *n*-alkylphenols exhibit the same germicidal activity, the ortho ethers are definitely less effective than the meta- and para-compounds. On the other hand, the maximum effect against *B. typhosus* in each series occurs at the five-carbon-atom side chain, as with the alkylphenols. Guaiacol and its derivatives have been used medicinally for some time. In the older literature (50) it was described as being a stronger disinfectant than phenol. A series

of 4-alkylguaiacols was found to have its maximum phenol coefficient at the *n*-amyl compound (63), but the value was low. The condensation product of 2-ethyl-1-butanol and guaiacol showed phenol coefficients of 73 and 63 against *Staph. aureus* and *E. typhi*, respectively (59a). The preparation of mono ethers of 4-chlororesorcinol has been described in the patent literature (122). These products are probably mixtures of isomers.

The effect of a second ether oxygen in a phenol side chain is to reduce the bactericidal action still further (20, 65). This is shown in table 19. Ap-

TABLE 18
Phenol coefficients at 37°C. of isomeric hydroxyphenyl alkyl ethers

ALKYL GROUP	PHENOL COEFFICIENT AGAINST <i>B. typhosus</i>			PHENOL COEFFICIENT AGAINST <i>Staph. aureus</i>		
	Para-compound	Meta-compound	Ortho-compound	Para-compound	Meta-compound	Ortho-compound
None	>12.	0 4	0 87	0.44	0 4	0.58
Methyl	1 0	1 5	0.91	0 8	1.2	0 73
Ethyl	1 5	3 6	1.8	1.5	3 0	1.6
<i>n</i> -Propyl	5 4	6 9	4.1	4 1	5 4	3 8
<i>n</i> -Butyl	14.	20.	9.8	9.	18.	10.
<i>n</i> -Amyl	29	38.	22	30.	36	23.
<i>n</i> -Hexyl	18	46	17	100	125.	28.
<i>n</i> -Heptyl	17	21	9 7	200	330	37.
<i>n</i> -Octyl		2 3		360	580.	
<i>n</i> -Nonyl		3.4			650.	
<i>sec</i> -Amyl	19	(26)		26.	(31)	
Cyclohexyl		(18)			(20)	

TABLE 19
Phenol coefficients of resorcinol monoethers

ETHER	PHENOL COEFFICIENT	
	<i>B. typhosus</i> (20°C)	<i>Staph. aureus</i> (37°C.)
β -Ethoxyethyl	2.5	5
β -Butoxyethyl	10.	5
γ -Butoxypropyl	6.	5

parently no alkoxyalkylphenol has been studied, so the effect of an ether linkage in the side chain where the oxygen is not attached to the nucleus is not known. One or more alcohol groups in the side chain of an aryl alkyl ether reduces the phenol coefficient to zero (118). Against *Staph. aureus* at 37.5°C. the monoallyl and diallyl ethers of pyrogallol (66) have the phenol coefficients 15 and 6, respectively. Information is not available on other unsaturated ethers.

The results obtained with hydroxyphenyl alkyl ethers made it of interest to determine the germicidal activity of the corresponding sulfides. It is brought out in table 20 that, for the lower members of the series against *B. typhosus*, the sulfides are about five times as effective as the ethers (95, 97, 100, 147). Separating the alkyl group from the phenol nucleus by sulfur results in a rise in the phenol coefficient. The *p*-hydroxy sulfides are more potent than the meta- and ortho-isomers. For the *n*-butyl com-

TABLE 20
Phenol coefficients of p-hydroxyphenyl alkyl sulfides and ethers

ALKYL GROUP	PHENOL COEFFICIENT AGAINST <i>B. typhosus</i>		PHENOL COEFFICIENT AGAINST <i>Staph. aureus</i>	
	Sulfide	Ether	Sulfide	Ether
Methyl.	5	1	4 (8)	0.8
Ethyl	12	1.5	12 (10)	1.5
<i>n</i> -Propyl	25	5.4	25 (36)	4.1
<i>n</i> -Butyl	75	14	60 (77)*	9.3
<i>n</i> -Amyl.	75	29	150 (120)	30.
<i>n</i> -Hexyl	40	18	200 (230)*	100.
Isopropyl			(20)	
Isobutyl			(61)	
Isoamyl.			(30)	
Benzyl			(20)	

* Here the solvent was 0.01 *N* sodium hydroxide.

TABLE 21
Phenol coefficients of 3-methyl-4-hydroxyphenyl alkyl sulfides

ALKYL	PHENOL COEFFICIENT		
	<i>B. typhosus</i>	<i>Staph. aureus</i>	<i>Strep. hemolyticus</i>
Methyl	13	12	10
Ethyl	20	50	40
<i>n</i> -Propyl	23	80	80
<i>n</i> -Butyl	14	100	80
<i>n</i> -Amyl.	8	250	200

pounds the phenol coefficient decreases from 77 to 40 to 25 as the hydroxyl group approaches the sulfur atom. Introduction of a methyl group into the aryl nucleus decreases greatly the maximum effectiveness of the sulfides against *B. typhosus* (150). The results are shown in table 21.

A series of 3-chloro-4-hydroxyphenyl alkyl sulfides has been prepared (28), but unfortunately no information is available concerning their bactericidal properties. The first attempts to prepare dihydroxyphenyl alkyl

sulfides were unsuccessful (148), but more recently (149) a series of 3,5-dihydroxy compounds has been synthesized by a series of reactions starting with benzenetrisulfonic acid. The phenol coefficients for these compounds are much lower than was anticipated. They are listed in table 22. Monoethers of phloroglucinol are not available for comparison, but the *sym*-alkylresoreinols mentioned earlier did not show abnormally low germicidal activity.

XII. HYDROXY DERIVATIVES OF BIPHENYL

Although there are many claims in the patent literature and some general statements elsewhere to the effect that hydroxybiphenyls are excellent bactericidal agents, the quantitative information available is small. Harris and Christiansen (56) have shown that 2-hydroxybiphenyl kills *B. typhosus* in a dilution of 1:2000 in 5 min., while against *Staph. aureus* the dilution necessary is 1:800. Woodruff gives the phenol coefficient here as 12.5

TABLE 22

Phenol coefficients of 3,5-dihydroxyphenyl alkyl sulfides at 37°C.

ALKYL GROUP	PHENOL COEFFICIENT	
	<i>B. typhosus</i>	<i>Staph. aureus</i>
Methyl	5.9	4 3
Ethyl	8 3	5 2
<i>n</i> -Propyl	16.	11.
<i>n</i> -Butyl	17.	14.
<i>n</i> -Amyl	<16.	<11
<i>n</i> -Hexyl	<34	<23.

(162). A study of the action of the same compound upon *Mycobacterium tuberculosis* in soap solutions (157) showed it to be lethal after 2 min. in 0.5 per cent concentration in a 1 per cent soap solution; with a 0.5 per cent soap solution, the presence of 0.25 per cent of the phenol was effective in 30 min., while at lower concentrations of soap and the phenol, the results were uncertain. Fuller (52) has reported that *o*-hydroxybiphenyl has remarkable penetrating power on the intact skin and is destructive toward the Streptococcus and Staphylococcus groups of organisms, but no numerical data were given.

A recent (104) determination of the behavior of 3-hydroxybiphenyl toward *Staph. aureus* gave a phenol coefficient of 33, indicating that phenyl is somewhat less effective than *n*-butyl in increasing the germicidal action of phenol. The activity toward *B. typhosus* is only about one-fifth as great (56). Against *Staph. aureus*, *p*-phenylphenol is even less effective than the ortho-isomer (162). Tests made with 2,4-dihydroxybiphenyl

(146) against *Staph. aureus* at 37°C. gave a phenol coefficient of 14, while the 3,5-isomer has a value of less than 12. 2,5-Dihydroxybiphenyl requires a dilution of 1:500 to kill either *B. typhosus* or *Staph. aureus* in 5 min. (56), while for the 3,4-isomer the dilutions were 1:2000 and 1:1200, respectively.

Halogen derivatives of hydroxybiphenyls have been described several times in the patent literature as having bactericidal properties (17, 32, 33, 69, 143). *o*-Bromo-*p*-phenylphenol has a phenol coefficient of 62.5 against *Staph. aureus* at 37°C. (162). A variety of alkylhydroxybiphenyls has also been investigated (16, 29, 33, 56). Against *Staph. aureus*, 3-alkyl-2-hydroxybiphenyls are less active than 2-hydroxybiphenyl itself, while the 5-alkyl isomers are more effective, reaching a maximum with the *n*-propyl derivative. Against *B. typhosus*, both groups of alkyl compounds exhibit decreased bactericidal properties. In general, the regularity in the effect of the alkyl group shown in the simple alkylphenols is no longer in evidence. Many halogenated (15) alkylhydroxybiphenyls have been listed as of potential value as germicides.

XIII. HYDROXY DERIVATIVES OF DIPHENYLMETHANE

It would be of particular interest to be able to compare a series of hydroxybiphenyl derivatives with the corresponding diphenylmethanes, where the two aromatic nuclei are separated by a saturated group which prevents resonance effects from being transferred from one ring to the other. Owing to Klarman and coworkers (76, 78, 83), information is available for a variety of mono- and di-hydroxydiphenylmethane derivatives. The monohydroxy compounds are listed in table 23. Huston and coworkers (68) have prepared many other benzylphenols.

Several conclusions are obvious from these data. As for the simple halogenated phenols, a halogen ortho to a hydroxyl group increases the phenol coefficient less than when in the para-position. On the whole, it makes little difference which ring bears the halogen atom so long as it is not ortho to the hydroxyl. Multiple substitution of methyl and halogen groups produces substances highly active against *Staph. aureus* and *Strep. hemolyticus*.

A few derivatives of 2,4-dihydroxydiphenylmethane have been investigated. Their relative effectiveness is indicated in table 24. Here a halogen in the second ring raises the bactericidal value more than one adjacent to hydroxyl. The unsubstituted compound has about the same bactericidal action as 2,4-dihydroxybiphenyl (146).

An interesting series of diphenylmethane derivatives has been prepared (55) by condensing 2-hydroxy-3,5-dibromobenzyl bromide with various phenols, giving products having one or more hydroxyls in each ring.

TABLE 23

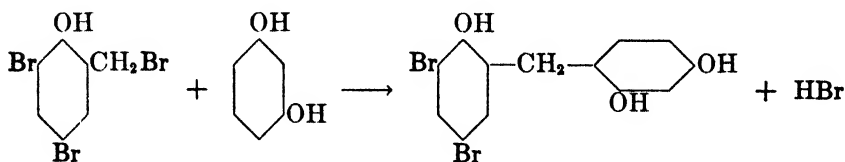
Phenol coefficients of hydroxydiphenylmethane derivatives

SUBSTITUTED HYDROXY COMPOUND	PHENOL COEFFICIENT			
	<i>E. typhi</i>	<i>E. paratyphenteriae</i>	<i>Staph. aureus</i>	<i>Strep. hemolyticus</i>
3-Chloro-4-	36	54	125	165
3-Chloro-2-	24	36	71	94
5-Chloro-2-	74	92	215	245
4'-Chloro-4-	83	98	170	165
4'-Chloro-2-	57	90	190	175
3-Bromo-4-	19	37	170	185
5-Bromo-2-	26	55	295	310
3,4'-Dichloro-4-	41	110	345	175
3,5-Dichloro-2-	22	85	65	250
3-Chloro-4'-bromo-4-	17	24	200	565
5-Chloro-3-methyl-2-	16	25	245	300
4'-Chloro-3-methyl-2-	16	26	240	260
5-Chloro-4-methyl-2-	17	34	405	455
5-Chloro-4,6-dimethyl-2-	31	22	920	785
4'-Bromo-4,6-dimethyl-2-			420	600
5 - Chloro - 3 - isopropyl - 6 - methyl-2-	16	15	35	38

TABLE 24

Phenol coefficients of substituted 2,4-dihydroxydiphenylmethanes

SUBSTITUENT	PHENOL COEFFICIENT	
	<i>B. typhosus</i>	<i>Staph. aureus</i>
None ..	18	11
5-Chloro-	48	37
4'-Chloro-	63	40
5-Bromo-	37	45
4'-Bromo-	55	51



In table 25 the compounds made in this manner are listed, together with their "maximum killing dilutions" at 5 min. The first column indicates the phenol used in the condensation. The determinations were made at 37°C. by the F.D.A. method. The presence of a hydroxyl group

in each nucleus apparently reduces the effect toward *E. typhi* to a small value, whereas some of the compounds are quite effective against *Staph. aureus*.

When two aryl groups, one of them containing one or more hydroxyl groups, are separated by more than one methylene group, the germicidal power rises and then falls (74). This is brought out in table 26.

TABLE 25

Bactericidal properties of 2-hydroxy-3,5-dibromodiphenylmethane derivatives

PHENOL	EFFECTIVE DILUTION	
	<i>E. typhi</i>	<i>Staph. aureus</i>
Phenol	1:500	1:1200
2,4-Dibromophenol	1:400	1:3000
2,4-Diiodophenol	1:600	1:5000
Resorcinol	1:200	1:250
Dibromoresorcinol	<1:100	1:300
<i>m</i> -Cresol	1:600	1:2000
Dibromo- <i>m</i> -cresol	1:200	1:8000
<i>o</i> -Cresol	1:200	1:1000
Bromo- <i>o</i> -cresol	1:500	1:5000

TABLE 26

Phenol coefficients of aralkylphenols

COMPOUND	PHENOL COEFFICIENT AGAINST <i>B. typhosus</i>
<i>p</i> -Benzylphenol	4.6
4-Benzylresorcinol	22.
4-Phenethylresorcinol	40.
4-Phenpropylresorcinol	31.
Benzylphloroglucinol	7.5
Phenethylphloroglucinol	8.
Phenpropylphloroglucinol	8.8

In the early investigations of Ehrlich and coworkers (11, 12), it was found that when two benzene rings, one bearing a hydroxyl group, were separated by the ketone, sulfone, or carbinol grouping, the bactericidal properties of the compound were reduced to a low value compared with the diaryl-methane derivative.

XIV. HYDROXY DERIVATIVES OF ARYL ETHERS AND OF ARYL SULFIDES

It has been pointed out earlier that the bactericidal efficiency of hydroxy-phenyl alkyl sulfides is much greater than that of the corresponding ethers.

It is therefore of interest to compare hydroxy derivatives of purely aromatic sulfides with the oxygen compounds. Although the sulfides were found to be more effective (62, 72), the difference is much smaller than in the case of the alkyl aryl compounds. This is brought out in table 27. Walter (161) has extended this comparison for the *p*-hydroxy compound to the

TABLE 27

Phenol coefficients for hydroxy aryl ethers and sulfides against B. typhosus

POSITION OF HYDROXYL	PHENOL COEFFICIENT	
	Ether	Sulfide
Ortho	17	33
Meta	40	68
Para	41	115

TABLE 28

Phenol coefficients of hydroxy diaryl compounds against Staph. aureus

<i>p</i> -HYDROXY DERIVATIVE OF	PHENOL COEFFICIENT
Phenyl ether	40
Phenyl sulfide	100
Phenyl selenide	100
Diphenylamine	10
Diphenylmethane	100

TABLE 29

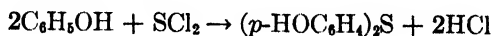
Phenol coefficients of phenols and corresponding aryl sulfides against Staph. aureus in 30 per cent alcohol

PHENOL	PHENOL COEFFICIENT OF PHENOL	PHENOL COEFFICIENT OF SULFIDE	RATIO
Phenol.	1	12 5	12 5
Resorcinol	0 8	3 0	4
<i>m</i> -Cresol	2.1	17	8
<i>p</i> -Chlorophenol.	6 2	63	10
<i>p</i> -Bromophenol.	4 2	42	10
Thymol	8.3	83	10

selenide, amine, and methane derivatives. In table 28 are shown the results obtained with *Staph. aureus* by the Rideal-Walker technic. The hydroxy ethers are of no value as urinary antiseptics because they are excreted as esters of sulfuric acid. *p*-Hydroxyphenyl *p*-tolyl sulfide has been patented as a germicide (73). Iodine derivatives of hydroxyaryl

sulfides are too insoluble to produce a bactericidal effect in the usual tests (9).

An interesting series of sulfides has been prepared by the action of sulfur dichloride upon phenols (45).



These compounds, as shown in table 29, are about ten times more effective as bactericidal agents than the original phenols, except in the case of resorcinol; however, 0.8 is about twice the figure found for the phenol coefficient of resorcinol by other investigators (75).

XV. DISCUSSION ✓

On the basis of the evidence presented, a number of generalizations can be formulated.

1. Whatever the reasons for the lack of agreement of the phenol coefficients reported by different investigators for the same compound, it is evident that any conclusion drawn from a comparison of data on different substances when the data are not from the same laboratory must be accepted with reservations. On the other hand, a general trend in bactericidal activity shown in a series of compounds can be relied upon to repeat itself in different sets of data, even though the numerical values of the phenol coefficients may vary.

2. It is clear that the phenol coefficient of a compound must depend upon several properties, which may be physical or chemical or both. A property which is dominant in determining bactericidal activity toward *B. typhosus*, for example, must be relatively unimportant with another type of organism such as *Staph. aureus*. Otherwise the variation in effectiveness toward different organisms shown in a homologous series becomes unintelligible. The use of phenol as a reference standard somewhat clouds the issue here. A compound may have the same phenol coefficient against two types of organisms and still differ markedly in its relative lethal effects because of the difference shown by phenol in its action on the same two types of organism. In other words, the mechanism of bactericidal action must, for the time being, be considered as a separate problem for each type of organism.

3. The introduction of halogen into the nucleus of a phenolic compound without exception increases its bactericidal potency. This increase is less for the ortho-position than the para, perhaps owing to interaction between the hydroxyl groups and halogen atoms. Little evidence is available for meta-compounds. The effect of halogen substitution, in general, increases with increasing atomic weight of the halogen. However, the effect of iodine has been little studied.

4. The introduction of an alkyl group into the phenol nucleus produces a rise in bactericidal action, followed by a decrease as the carbon chain extends beyond five or six carbon atoms when *B. typhosus* and other Gram-negative organisms are employed for testing. For *Staph. aureus* and other Gram-positive varieties, the increase in activity continues somewhat irregularly until the compound becomes too insoluble to test satisfactorily. A normal carbon chain has more effect than a branched one containing the same number of carbon atoms. A primary alkyl group has more effect than a secondary or tertiary alkyl group of the same weight. A given number of carbon atoms produces more effect in a single side chain than when distributed between two or more. The effect of halogen and alkyl on bactericidal properties is more or less independent, i.e., if an alkyl group raises the phenol coefficient a halogen atom increases it still further.

The effect of introducing an alkyl group into a hydroxybiphenyl or hydroxydiphenylmethane is irregular and unpredictable.

5. Separation of an alkyl group from the phenol nucleus by oxygen decreases the germicidal activity, and the presence of oxygen as an alcohol or ether group in the side chain likewise produces this effect. On the other hand, a sulfur atom between the aryl and alkyl group increases the bactericidal action, the sulfur acting somewhat as an additional methylene group.

6. Increasing the number of hydroxyl groups attached to an aromatic nucleus decreases the germicidal activity, a decrease that cannot effectively be compensated for by alkyl and halogen when more than two hydroxyl groups are present.

7. Conclusions about compounds containing two or more aromatic nuclei are subject to the limitations indicated under paragraph 1. It appears that separation of the benzene rings of hydroxybiphenyl by means of groups which do not increase water-solubility or are not strongly polar,—such as methylene, ethylene, possibly trimethylene, sulfur, and selenium,—either does not change or increases bactericidal activity. On the other hand, the effect of the ketone, sulfone, and carbinol groups is just the reverse. The effect of the ether linkage is small and uncertain as to direction. It would be expected that the oxygen of diaryl ethers would have very weakly basic properties and hence influence the water-solubility of these compounds to a relatively small degree. Little systematic information is available concerning hydroxy derivatives of fused ring systems.

Suggestions for further research

Two types of research problems are in need of investigation in the field of phenolic bactericidal agents. In the first place, there are a number of serious gaps in our knowledge of the effect of certain groups. A study of

the effect of halogen meta to hydroxyl would be of interest, as in the one known example the bactericidal value is much higher than for the other isomers. It would be of interest to know whether the effect of halogen in the ring of hydroxyphenyl alkyl sulfides is to increase germicidal action or to decrease it, as does a methyl group. The synthesis of halogenated hydroxybiphenyls in which the halogen is not in the same ring as the phenol group also seems justified, in order to be able to compare such compounds with the diphenylmethane derivatives already investigated. It seems likely that a study of certain naphthalene derivatives,—in particular, 1,3-dihydroxynaphthalene and its substitution products,—would yield results of at least theoretical importance.

The second type of problem to be suggested is that dealing with the mechanism of bactericidal action and the correlation of this action with something other than molecular structure. Data are needed on the physical properties of phenolic compounds; in particular, solubilities, surface tension or interfacial tension effects, the distribution ratio between immiscible solvents, and adsorption phenomena seem worthy of study. From a knowledge of these properties for a relatively few carefully selected compounds, some insight into the mechanism of bactericidal effects may be obtained.

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THE THEORY OF ABSOLUTE REACTION RATES AND ITS APPLICATION TO VISCOSITY AND DIFFUSION IN THE LIQUID STATE

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Received December 23, 1940

A summary of the formulas of statistical mechanics is given, followed by a discussion of potential-energy surfaces. This is used as a background for a discussion of the theory of absolute reaction rates. The formal equations for viscous flow in liquids are then developed, followed by a discussion of the evaluation of the quantities entering into the equations. Applications of the theory to viscous flow in normal liquids, associated liquids, and μ - and λ -sulfur are then described, as well as the effect of high hydrostatic pressure on viscosity. Diffusion in liquids is then treated as a rate process, and conclusions as to the nature of the process are drawn from inspection of the available data.

With the aid of the theory and generalizations discussed here it is possible from thermodynamic data to calculate the viscosity of any normal liquid to within a small factor at any temperature from its freezing point to its boiling point, and at any pressure from 1 to 10,000 atmospheres.

The treatment of the viscosity of liquids described here is based on the theory of absolute reaction rates developed to treat ordinary chemical reactions (13, 17, 18, 71). Since this theory is based on the concepts and equations of elementary statistical mechanics, a brief survey of the subject will be given at this point, followed by the development of the theory of absolute reaction rates. The applications which have been made to problems of viscous flow and diffusion will then be described.¹

¹ The symbols used in this paper are as follows:

a = activity

A = Helmholtz free energy

A, B, C = moments of inertia for a non-linear molecule

c = kinetic theory velocity

I. A BRIEF SURVEY OF STATISTICAL MECHANICS

Suppose that we have a system, two of whose energy levels are ϵ' and ϵ . The ratio of the probabilities, p'/p , of the system being in these two states is given by the Boltzmann equation (24):

$$p'/p = \exp(-(\epsilon' - \epsilon)/kT) \quad (1)$$

where k and T are, as usual, the gas constant (Boltzmann) and the absolute temperature, respectively. Subject to the restriction imposed by the energy difference, all states are taken to be equally probable. In quantum statistics, different states of the system are distinguished not by different energies but by different solutions of ψ in the wave equation

$$\frac{\hbar^2}{8\pi^2m_e} (\partial^2\psi/\partial x_i^2 + \partial^2\psi/\partial y_i^2 + \partial^2\psi/\partial z_i^2) + (E - V)\psi = 0 \quad (2)$$

Thus, two *different* states may have the *same* energy and, according to our postulate, should therefore be equally probable. The number of states having a certain energy is called the *a priori* probability or statistical weight and is designated by the symbol ω .

With the aid of the Boltzmann factor, one may readily obtain expressions for the thermodynamic properties of the system. Let a be the probability

C = concentration

C_p = specific heat at constant pressure

C_v = specific heat at constant volume

D = diffusion constant

d = diameter

E = energy

F = partition function with the volume divided out, Gibbs free energy

f = partition function per system; force

H = Hamiltonian, heat content

h = Planck's constant

I = moment of inertia for a linear molecule

j = quantum number for rigid rotator

K = equilibrium constant; thermal conductivity

k = Boltzmann's constant

k = rate constant

l = length

M = molecular weight

m = mass of a molecule

N = Avogadro's number; mole fraction

n = quantum number for particle in a box and harmonic oscillator; number of molecules per cubic centimeter

P = pressure

p = probability; momentum

q = generalized coordinate; \dot{q} = generalized velocity

R = gas constant

that the system will be in the state of lowest energy. Then the ratio of the probability of the system being in any other state to the probability that it will be in the lowest state is given by equation 1. Since the sum of the probabilities that the system will be in some one of the states is unity, we have

$$1 = a\omega_0 + a\omega_1 \exp(-\epsilon_1/kT) + a\omega_2 \exp(-\epsilon_2/kT) + \dots$$

$$= a \sum_i \omega_i \exp(-\epsilon_i/kT) \quad (3)$$

or

$$1/a = \sum_i \omega_i \exp(-\epsilon_i/kT) \quad (4)$$

where ϵ_i is the energy of the system in the i^{th} state, referred to its energy in the lowest state. The average energy, $\bar{\epsilon}$, in excess of that in the lowest state, is seen to be

$$\bar{\epsilon} = a\omega_0\epsilon_0 + a\omega_1\epsilon_1 \exp(-\epsilon_1/kT) + a\omega_2\epsilon_2 \exp(-\epsilon_2/kT) + \dots \quad (5)$$

$$\bar{\epsilon} = a \sum_i \omega_i \epsilon_i \exp(-\epsilon_i/kT) = \frac{\sum_i \omega_i \epsilon_i \exp(-\epsilon_i/kT)}{\sum_i \omega_i \exp(-\epsilon_i/kT)} \quad (6)$$

- r = radius
 S = entropy
 T = absolute temperature; kinetic energy
 t = Centigrade temperature
 u = velocity of sound
 V = molal volume; potential energy
 v = volume per molecule; velocity
 V_f = free volume per mole
 v_f = free volume per molecule
 x, y, z = Cartesian coördinates

 α = accommodation coefficient
 β = compressibility ($= -(1/V)(\partial V/\partial P)_T$)
 γ = ratio of C_p to C_v ; activity coefficient
 ϵ = energy level
 η = viscosity
 Θ = Einstein characteristic temperature
 κ = partition function for a crystal; force constant; transmission coefficient
 λ = dimension of a molecule
 μ = reduced mass
 ν = frequency
 σ = symmetry number; collision diameter
 φ = fluidity ($= 1/\eta$)
 ψ = wave function
 ω = statistical weight

The summation giving $1/a$ (equation 4) is called the partition function of the system and is designated by the symbol f . Using this notation, equation 6 becomes

$$\bar{\epsilon} = kT^2 d(\ln f)/dT \quad (7)$$

If we are concerned with a large number, N , of systems,² the energy is N times that for a single one. Thus, if we wish to know the total energy of a mole of dilute benzene vapor, and f is the partition function of a single molecule, the required value is given by

$$\bar{E} = NkT^2 d(\ln f)/dT = kT^2 d(\ln f^N)/dT \quad (8)$$

This expression for the energy, \bar{E} , in terms of the partition function, can be related to the Helmholtz free energy, A , by the thermodynamic relation,

$$\bar{E} = E = -T^2 d(A/T)/dT \quad (9)$$

where E is the usual thermodynamic energy.

For these two expressions to be consistent we must have

$$A/T = -k \ln f^N + \text{constant} \quad (10)$$

The value of the constant is found by the third law of thermodynamics or by quantum mechanics to be equal to the logarithm of factorial N ($\ln N!$), where N is the number of identical systems in the assembly. If the systems are identical, and if one uses quantum mechanics to solve for the allowed energy levels of the ensemble, one would then find directly for the partition function, $\ln (f^N/N!)$. In this case the $N!$ enters because the Pauli exclusion principle allows only eigenfunctions which are anti-symmetric in the N identical systems (9, 50). This treatment then gives

$$A/T = -k \ln f^N + \ln N! \quad (11)$$

In order to simplify this formula, Stirling's approximation (8) may be used.

$$\ln N! = N \ln N - N \quad (12)$$

Substitution of equation 12 into equation 11 gives

$$A = -RT[\ln (f/N) + 1] \quad (13)$$

a formula which, from the nature of its origin, must be valid for gases.

² The word "system" is used here in the same sense as Fowler (24) employs it, to mean something which possesses a set of energy levels. This may be a single oscillator, a molecule, an entire crystal, or a liquid. "Assembly" is the term employed to denote a large number of systems whose energy levels are coupled by an amount sufficient to permit exchange of energy but insufficient to cause these levels to be different from those for the isolated system.

The procedure for crystals is to calculate the partition function κ for the N atoms or molecules as a single system so that, in this case, one has for the Helmholtz free energy

$$A = -kT' \ln \kappa \quad (14)$$

Having expressions for the free energy and the energy, the entropy in terms of the partition function follows directly from the thermodynamic equation,

$$A = E - TS \quad (15)$$

Substituting equation 13 or 14 and equation 8 into equation 15, we obtain

$$S = R[\ln (f/N) + 1] + \bar{E}/T \text{ (gas)} \quad (16)$$

and

$$S = k \ln \kappa + \bar{E}/T \text{ (crystal)} \quad (17)$$

The equation of state also follows from the partition function by means of the equation,

$$P = -(\partial A/\partial V)_\tau \quad (18)$$

A. Explicit expressions for the partition functions

Thus far, the partition function has been defined in a perfectly general way in terms of the energy levels. However, the energy levels vary in a simple fashion for many systems met with in physical problems, so that the sums can be put into more convenient forms.

The energy levels obtained by the aid of equation 2 for a particle moving in a cubical box of edge l and possessing kinetic but not potential energy are

$$\epsilon_n = \frac{h^2(n_x^2 + n_y^2 + n_z^2)}{8ml^2} \quad (19)$$

where the n 's are integers. The partition function defined by equation 4 is found to be

$$f = (2\pi mkT/h^2)^{3/2} l^3 \quad (20)$$

when the summation is approximated by an integration. The error introduced by this approximation is inappreciable, except at very low temperatures or high pressures. Even for the extreme case of hydrogen molecules at 1°K. enclosed in a box of 10^{-2} cm. (neglecting molecular interaction as we have done), the error made is only 1 in 10^5 in f (32).

The energy levels for the harmonic oscillator are $\epsilon_n = (n + 1/2)h\nu$, and the partition function for a single oscillator of this type is

$$f_v = [1 - \exp(-h\nu/kT)]^{-1} \quad (21)$$

The rigid linear rotator has energy levels,

$$\epsilon_j = \frac{h^2(j)(j+1)}{8\pi^2 I} \quad j = 0, 1, 2, \dots \quad (22)$$

each energy level being $(2j + 1)$ -fold degenerate, and the corresponding partition function is closely approximated by

$$f_R = 8\pi^2 I kT / \sigma h^2 \quad (23)$$

Here I is the moment of inertia, and σ is the symmetry number. For a diatomic molecule composed of two like atoms, σ is equal to 2. The quantum mechanics of the three-dimensional rotator with three unequal moments of inertia has not been completely solved, but the classical integration over phase space (a procedure exactly analogous to taking the sum (4), except that the energy levels are considered to be continuous rather than discrete) yields

$$f_R = 8\pi^2 (8\pi^3 k^3 T^3 ABC)^{1/2} / \sigma h^3 \quad (24)$$

where A , B , and C are the three principal moments of inertia.

The division of the complete partition function into factors corresponding to translational, rotational, and vibrational terms is valid only in case there is no interaction between these various degrees of freedom. In that case the energy levels can be expressed as sums of terms, and the partition function corresponding to each degree of freedom can be factored out. In making computations of the thermodynamic properties of gases from spectroscopic data, interaction terms between rotational and vibrational energy levels are often included.

B. Classical integration over phase space

It will be useful at this point to inquire into the relationship of the above formulae to those obtained by the use of classical statistics.

The classical analog of the quantum-mechanical sum of states is defined by

$$f = (1/h^N) \int \int \int \dots \int \int \int \exp(-H/kT) dp_1 \dots dp_n, dq_1 \dots dq_n \quad (25)$$

where the integration is to be taken over the whole of momentum coordinate phase space and H is the classical Hamiltonian, defined by

$$H = T + V \quad (26)$$

where T is the kinetic energy, and V is the potential energy of the assembly. For a single particle, T is given by

$$T = (1/2)mv^2 \equiv (1/2m)(p_x^2 + p_y^2 + p_z^2) \quad (27)$$

p_x , p_y , and p_z being the momenta along the three axes.

For a free particle, V is equal to zero, and equation 25 becomes

$$f = (1/h^3) \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} \int_{-\infty}^{+\infty} [\exp(-1/2mkT) \cdot (p_x^2 + p_y^2 + p_z^2)] dp_x dp_y dp_z \\ \times \int_0^{l_x} \int_0^{l_y} \int_0^{l_z} dx dy dz \quad (28)$$

$$f_T = (2\pi mkT/h^2)^{3/2} V \quad (29)$$

since the volume V is given by the product $l_x l_y l_z$.

The classical partition function for the one-dimensional simple harmonic oscillator may be obtained from equation 25 by direct integration, recalling that

$$H = (1/2m)(p_x^2) + (1/2)\kappa x^2 \quad (30)$$

and that the frequency, ν , is related to the force constant, κ , by the equation,

$$\nu = (1/2\pi)(\kappa/m)^{1/2} \quad (31)$$

The expression obtained for the partition function,

$$f_v = kT/h\nu \quad (32)$$

may also be obtained from equation 21 by expanding the exponential in powers of $h\nu/kT$ and dropping all but the first two terms. Equation 32 is thus seen to be a good approximation only if $h\nu/kT$ is small.

The classical partition function for the two-dimensional rigid rotator is the same as that already obtained (equation 23) by replacing the summation over states by an integration.

II. THEORY OF ABSOLUTE REACTION RATES

A. Potential-energy surfaces (22, 24, 43, 44)

The statistical treatment of reaction rates involves the concept of a potential-energy surface giving the potential energy of the reacting molecules as a function of their position, orientation, and interatomic distances. Such surfaces giving the potential energy at every point in configuration space are generally drawn as contour graphs, each line on the graph being a line of equal potential energy, called an equipotential line. If a complete surface is considered, extending all the way from the stable reactants to

the stable products, it will be seen that there is an "easiest reaction path" between the two, i.e., a path drawn such that the potential energy at all points along the line increases in a direction at right angles to the line. The velocity with which the reaction proceeds depends on the temperature, on the difference in energy between the initial state and the point of highest energy on the easiest reaction path, and on other factors which are discussed below. Most of the thousands of compounds which are known are thermodynamically unstable; their apparent stability depends on the height of the easiest path that separates them from their more stable reaction products. On the basis of this picture, the existence of thermodynamically unstable compounds and their reaction to form more stable products is easy to understand. The compounds correspond to potential energy valleys separated from their possible reaction products by passes which have potential energy mountains on either side.

In general, $3n$ coordinates are required to describe the system of n atoms that are taking part in the reaction. But since the position of the center of gravity of the system is irrelevant, and since the potential energy of the system is practically independent of the rotation of the complex as a whole, the number of coordinates required to define the potential-energy function and the essential details of the relative motion reduces to $3n - 6$ or $3n - 5$, depending on whether the configuration is non-linear or linear.

Such a surface is illustrated in figure 1 for a simple three-atom reaction, the exchange of hydrogen for hydrogen in the para-hydrogen molecule to form an ortho-hydrogen molecule and a hydrogen atom. The surface is drawn for a linear triatomic molecule and should thus require $3 \times 3 - 5 = 4$ coordinates. However, this is reduced to two if the two degrees of freedom corresponding to bending vibrations are excluded. The axes are inclined at an angle of 60° to each other in order that the representation of the potential energies of various configurations of the system of three atoms will have exactly the same variation of its coordinates with time as a single point moving in the potential field with the inclined axes would have. Stated in another way, the particular manner of construction that is used eliminates cross terms from the potential function. The method of determining the effective mass of the single particle and the correct inclination of the axes to each other has been given by Wigner. The result³ is quoted by Eyring and Polanyi (22).

In terms of this surface the reaction is pictured as taking place as follows: As the hydrogen atom approaches the molecule of para-hydrogen, the bond between the hydrogen atoms weakens, and at the top of the easiest pass the configuration of lowest energy is one in which the three atoms

³ Because of an error in sign, the angle between the axes was originally given as 120° rather than 60° .

are arranged in a linear manner with the distances between neighboring atoms slightly greater than the distance in the normal hydrogen molecule. The potential energy is seen to become greater as the reactants approach each other, reaching a maximum value at two points where the configuration is intermediate between that of the reactants and products, and becoming less again as the products are formed. The point of highest energy on the easiest path from reactants to products is known as the activated state, and the configuration of the atoms at this point is called the activated complex. Owing to the symmetry of the reaction we are discussing here,

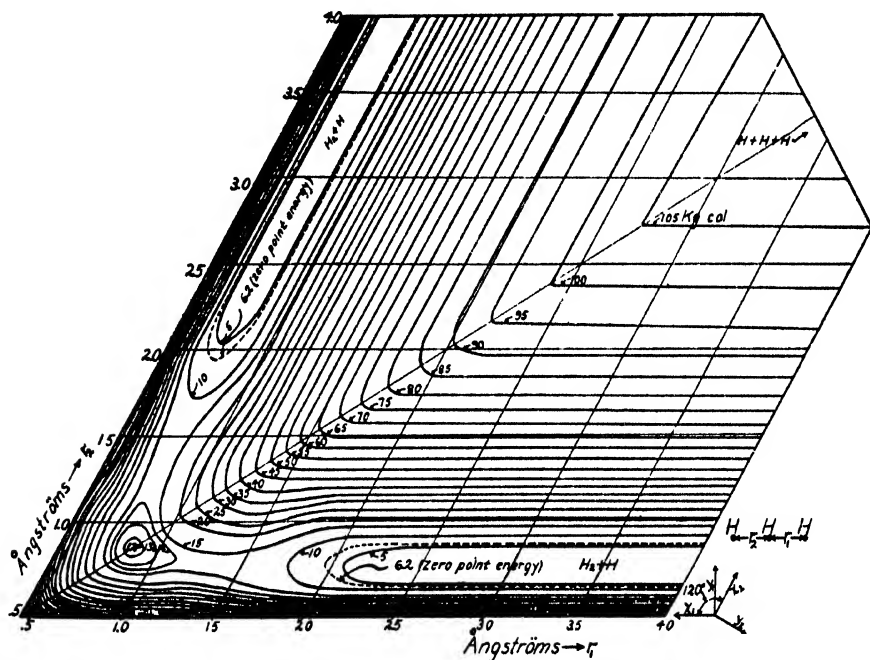


FIG. 1. Potential energy surface for three hydrogen atoms arranged in a line (20)

there are two symmetrical saddle points along the easiest path which are separated by a potential-energy basin. The presence of two saddle points along the easiest path is of importance in treating the transmission coefficient κ , which will be discussed in the next section.

The important point is that the properties of an activated complex are just those of an ordinary molecule except in the one degree of freedom along the easiest path, i.e., normal to the barrier. This becomes clear when it is recalled that by definition the activated complex is the configuration corresponding to the highest point (or points) along the easiest reaction path.

Thus the system is in stable equilibrium with respect to small displacements in every direction except the one normal to the barrier. The theory of small vibrations leads to a set of frequencies in exactly the same way as for an ordinary molecule, except that the square of the frequency for the degree of freedom normal to the barrier comes out with a negative instead of a positive sign, and hence from equation 31 it has a negative force constant. Figure 2 shows the normal modes of vibration for a symmetrical linear triatomic molecule. The one marked A has the negative force constant, the force constants for the normal modes B, C, and D being positive. The frequencies C and D are equal and correspond to a bending vibration in planes perpendicular to each other.

The mechanism described here for the reaction of $\text{H} + \text{H}_2(\text{para})$ has the same general features as that of any other chemical reaction having an activation energy. There is always an easiest reaction path between

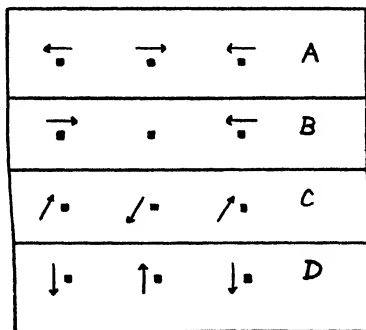


FIG. 2. Normal modes for a linear symmetrical triatomic molecule

reactants and products, and one or more saddle points along that path. The configuration at the highest saddle point is called the activated complex, which has the characteristics discussed above. Of course, the *actual* reaction paths will have a Boltzmann distribution about the *easiest* reaction path, and all these paths are included with their proper weight in the treatment of reaction rates by the ordinary statistical mechanical methods. Further, any rate process can be treated by the same general method. We now go on to develop the general equations which are applicable to any rate process.

B. Statistical formulation of reaction rate equations⁴

Equations giving the absolute rate of any rate process taking place on a single potential-energy surface may now be formulated in a perfectly

⁴ The formulation that we shall use is essentially the same as that given by Eyring (18). See, also, Pelzer and Wigner (51) for a treatment of reactions involving

general way as follows: The rate of reaction is given by the concentration of activated complexes, multiplied by the rate at which they decompose to form the reaction products. In general, the rate of reaction will be less than the rate of formation of activated complexes, since some of the activated complexes will return to the initial configuration without reacting. This can be expected to be the case in those very common instances where the easiest reaction path has two saddle points with a high-level basin lying between them. When there is no such high-level basin, the rate of reaction is simply the rate at which activated complexes reach the pass in such a direction that (regarded as classical particles) they can pass over and through it. When there is such a basin, the reaction rate is the rate

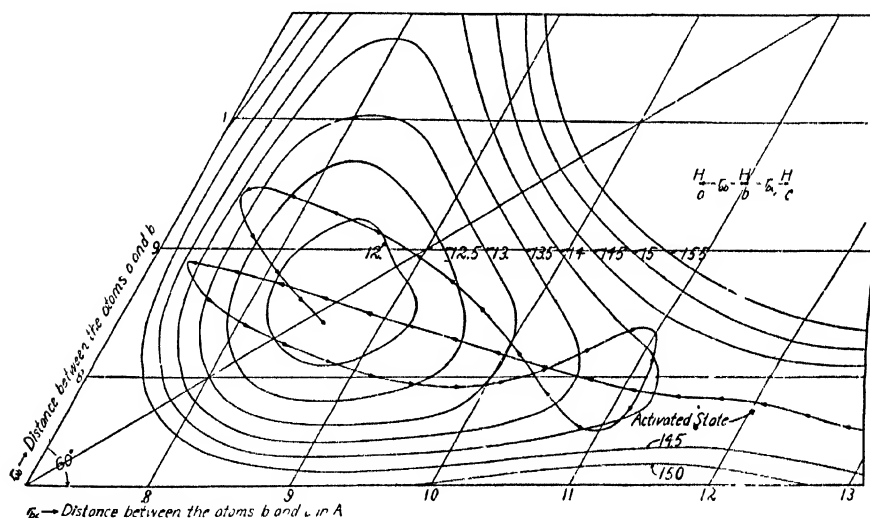


FIG. 3. The vibrational trajectory for linear H_3 , indicated by the line with arrows, was computed by employing the classical equations of motion (29).

at which activated particles pass into it, multiplied by the probability that the exit will be in a direction corresponding to reaction. This proba-

three atoms and Wigner (68) for a treatment of leakage and a quantum-mechanical extension of the earlier treatment. For the treatment from the quasi-thermodynamic point of view, see the papers of Evans and Polanyi (13) and Wynne-Jones and Eyring (71). A much earlier and interesting formulation of reaction rates by Marcelen (46) necessarily lacked the concept of the crossing of a potential barrier in configuration space which the London formulation suggested (44) and which led Eyring and Polanyi (22) to formulate explicitly the problem as the motion of a particle on a suitably constructed potential-energy surface. Further quantum-mechanical corrections are carefully considered in a recent article by Hirschfelder and Wigner (31) and are found to be, in general, small.

bility, called the transmission coefficient, is given the symbol κ . Figure 3 shows a trajectory of a particle following a linear vibrational distortion given it after it has passed the activated point into the high-level basin. The randomness of the path, combined with the fact that in this case the entry and exit are symmetrical, leads us to the conclusion that in this case κ must be very nearly equal to one-half. In other more complex cases the value of κ cannot be predicted exactly, but there is reason to believe that its value will generally not depart greatly from unity for the adiabatic reactions considered here.⁵ Another effect that may contribute to the rate is that of tunneling through the barrier, i.e., the reaction of molecules which do not possess the requisite energy. This effect has been considered by Wigner (31, 51, 68) and by Eckart (10). The correction term thus introduced is small for barriers of small curvature, but may become of importance for very thin barriers.

Then to the approximation that tunneling may be neglected, we may write for the specific reaction rate constant:

$$k = \kappa K^* \bar{p}/m^* \quad (33)$$

where m^* is the reduced mass of the activated complex, \bar{p}/m^* is its average velocity in the forward direction along the reaction path, and K^* is the equilibrium constant between activated complexes and reactants expressed in concentration units, per unit of length along the reaction path and per unit of volume, respectively. K^* may be written in terms of the partition functions of the activated complex and of the reactants. The expression for k then becomes:

$$k = \kappa (F^*/F_n) (\bar{p}/m^*) \quad (34)$$

or

$$k = \kappa (F^*/F_n) (\bar{p}/m^*) \exp(-E_0/RT) \quad (35)$$

where F^* is the partition function of the activated complex, and F_n represents that of the reactant molecules.

In equations 34 and 35 the F 's represent partition functions with the volumes divided out. Equation 34 differs from equation 35 only in that in the former the energy levels for both F^* and F_n must be referred to a common zero, while the latter refers the energy levels to the lowest state of the molecule to which the partition function applies. These equations are applicable to reactions in any phase. However, the partition func-

⁵ The term "adiabatic" is used here in Ehrenfest's sense to mean a reaction that takes place on a single potential surface in configuration space. Cases of reactions involving the transition of the system from one surface to another can also be treated by special methods. See Stearn and Eyring (62) and Evans, Eyring, and Kincaid (12).

tions vary with the phase in question. The equilibrium constant between reactants and the activated complex and therefore the rates in the liquid phase may be readily obtained from the corresponding expressions in the gas if vapor pressures are known. This is, of course, true of all equilibrium constants, and the calculations of the change of reaction rates and of equilibrium constants on taking systems from the gaseous to the liquid phase should ultimately be the most important chemical application of theories of liquids and solutions.

The average velocity, \bar{p}/m^* , with which the activated configuration travels over the barrier may be regarded as a pure translation for sufficiently flat surfaces, and its value is given in the usual way by the expression:

$$\frac{\bar{p}}{m^*} = \left[\int_0^\infty \frac{p}{m^*} \exp\left(-\frac{p^2}{2m^*kT}\right) dp \right] \left[\int_{-\infty}^{+\infty} \exp\left(-\frac{p^2}{2m^*kT}\right) dp \right]^{-1} \quad (36)$$

$$\frac{kT}{(2\pi m^*kT)^{1/2}}$$

However, the partition function for the activated complex per unit length along the reaction path contains the term $(2\pi m^*kT/h^2)^{1/2}$, so that the product of this term and \bar{p}/m^* equals kT/h . We then write as our final equation for k :

$$k = \kappa(kT/h)(F^\ddagger/F_n) \exp(-E_0/RT) \quad (37)$$

where E_0 is the energy of activation at the absolute zero and F^\ddagger is the partition function for the activated complex, taking the partition function for the degree of freedom in which the molecule is decomposing as equal to unity instead of $(2\pi mkT/h^2)^{1/2}$. It may be noted that F^\ddagger is formally the same as the partition function of a molecule exactly like the activated complex, with the degree of freedom normal to the barrier being a vibration sufficiently stiff for its partition function to reduce to unity. This idealized activated complex is a convenient concept for defining the free energy and entropy of activation in the usual way in terms of equilibrium constants. Thus,

$$\Delta F^\ddagger = -RT \ln K^\ddagger \quad (38)$$

and

$$\Delta F^\ddagger = \Delta H^\ddagger - T\Delta S^\ddagger \quad (39)$$

Using relations 38 and 39, the equation for k may be written in the equivalent forms,

$$k = (\kappa kT/h) \exp(-\Delta F^\ddagger/RT) \quad (40)$$

$$k = (\kappa kT/h) \exp(-\Delta H^\ddagger/RT) \exp(\Delta S^\ddagger/R) \quad (41)$$

These equations are not limited to the gaseous phase and may be applied to any rate process if the free energy of activation is known from any source.

A point of view which is less useful than that employed above, but which may aid in giving one an intuitive feeling for the significance of the factor kT/h in equations 37 to 41, is the following: The degree of freedom in which the decomposition is occurring has been regarded as a translation, but it may equally well be considered as a vibration. In this case the classical partition function is $kT/h\nu^*$. But the rate at which the decomposition is taking place must be equal to ν^* , the frequency of vibration along the coordinate normal to the barrier, since the force constant for this vibration is negative. That is, every vibration leads to decomposition. The product of $kT/h\nu^*$ and ν^* then gives kT/h as before.

In the use of equation 37 it should be emphasized that the partition functions contain all the energy levels which contribute at the temperature involved, i.e., excited electronic states should be included if they are not sufficiently high to be neglected.

C. Kinetic theory equations

Some of the simplest applications of the theory of absolute reaction rates are to be found in the derivation of ordinary kinetic theory formulae. The equations which are derived are, of course, identical with those secured by the ordinary methods, but the derivations are often simpler and clearly bring out the approximations which are made.

The rate at which molecules at a pressure P and temperature T strike a square centimeter of surface is found as follows: The activation energy for this process is equal to zero, and the activated complex is a molecule just breaking away from the wall with two degrees of translational freedom besides the one in which decomposition is occurring. κ is unity for such a process, and there is no possibility of leakage through a barrier, so that equation 37 reduces to

$$k = \frac{(kT/h)(2\pi m^* kT/h^2) F_R^\ddagger F_v^\ddagger}{(2\pi m kT/h^2)^{3/2} F_R F_v} \quad (42)$$

where F_R and F_v are rotational and vibrational partition functions, respectively. Since m^* , F_R^\ddagger , and F_v^\ddagger are the same for the activated complex as for the normal molecule, equation 42 may be simplified by cancellation to give

$$k = (kT/2\pi m)^{1/2} \quad (43)$$

The rate is given by the product, nk , where n is the number of molecules per cubic centimeter, so that equation 43 reduces to

$$\text{Rate} = nk = nkT/(2\pi m kT)^{1/2} = P/(2\pi m kT)^{1/2} \quad (44)$$

Another illustration which may be given is the calculation of the number of collisions between molecules in the gas phase. Consider first the case of the rate of collision between unlike molecules. In order to arrive at the conventional formula it is necessary to assume either that the colliding molecules are monatomic and hence have no rotational terms in their partition functions or that the rotations in the normal state are the same as those in the activated complex. The activated complex can be taken as the system of two molecules just breaking away from each other. We have thus:

$$F^\ddagger = (2\pi(m_1 + m_2)kT/h^2)^{3/2} 8\pi^2 I kT/h^2 \quad (45)$$

and

$$F_n = (2\pi m_1 kT/h^2)^{3/2} (2\pi m_2 kT/h^2)^{3/2} \quad (46)$$

and for the rate of collision, $Z_{1,2}$,

$$Z_{1,2} = n_1 n_2 k = n_1 n_2 \frac{kTF^\ddagger}{hF_n} \quad (47)$$

On substituting equations 45 and 46 into equation 47 and simplifying we arrive at

$$Z_{1,2} = \frac{2n_1 n_2 (2\pi kT)^{1/2} \sigma_{1,2}^2}{\mu^{1/2}} \quad (48)$$

where μ is the reduced mass defined by $\mu = m_1 m_2 / (m_1 + m_2)$, and $\sigma_{1,2}$ is the usual collision diameter. The collision diameter enters into the expression from the definition, $I = \mu \sigma_{1,2}^2$.

As a final illustration we give a derivation of the formula giving the number of collisions between unlike molecules having a relative velocity greater than a certain value. Consider the motion of two molecules of masses m_1 and m_2 having velocities along their line of centers \dot{q}_1 and \dot{q}_2 , respectively. Then the relative velocity, \dot{Q} , is defined by

$$\dot{Q} = \dot{q}_1 - \dot{q}_2 \quad (49)$$

The kinetic energy and momentum are given by

$$T = (1/2)m_1 \dot{q}_1^2 + (1/2)m_2 \dot{q}_2^2 \quad (50)$$

and

$$P = m_1 \dot{q}_1 + m_2 \dot{q}_2 \quad (51)$$

Since the motion of the center of gravity of the system may be taken equal to zero, we have

$$\dot{q}_1 = -m_2 \dot{q}_2 / m_1 \quad (52)$$

a relation which may be combined with equations 49 and 50 to give an expression for T in terms of \dot{Q} :

$$T = (1/2)\mu\dot{Q}^2 \quad (53)$$

Since this is the general expression for the kinetic energy relative to the line of centers, it must also be true at the point of collision. The rate expression may now be written

$$Z_{1,2} = \frac{n_1 n_2 (2\pi(m_1 + m_2)kT/h^2)^{3/2} (8\pi^2 I kT/h^2) (2\pi m^* kT/h^2)^{1/2} (\bar{p}'/m^*)}{(2\pi m_1 kT/h^2)^{3/2} (2\pi m_2 kT/h^2)^{3/2}} \quad (54)$$

where \bar{p}'/m^* is now not the over-all average velocity normal to the barrier, but the average velocity in the range of velocities we are considering. This value may be obtained by a procedure exactly analogous to that used in equation 36, except that the lower limit of integration is taken to be the minimum momentum P_0 instead of zero. This procedure gives

$$\bar{p}'/m^* = kT/(2\pi m^* kT)^{1/2} \exp(-P_0^2/2m^* kT) \quad (55)$$

Combining these last two expressions, we have

$$Z_{1,2} = 2n_1 n_2 (2\pi kT/\mu)^{1/2} \exp(-\mu\dot{Q}^2/2kT) \quad (56)$$

if we remember that in this case $m^* = \mu$.

It is interesting to note that if the term $\exp(-\mu\dot{Q}^2/2kT)$ had been regarded as an activation energy, we would have written down the expression 56 directly from the theory of absolute reaction rates. While the usual kinetic theory derivations of rate processes and those obtained by using the activated complex theory are interchangeable for simple atoms, only the latter theory is adequate for treating complicated molecules.

III. EQUATIONS FOR VISCOSITY AND DIFFUSION (19)

In this section we shall develop the formal equations for viscosity and diffusion, based on the following mechanism: Viscous flow is assumed to take place by the activated jumping of an aggregate composed of one or more molecules from an initial normal configuration to a second normal configuration. In common with chemical reactions, normal configurations are assumed to be separated by an intermediate, activated state corresponding to the activated complex. It is thus possible to use all the machinery developed in the preceding sections for the treatment of viscosity and diffusion. The magnitude of the free energy of activation and the exact mechanism for flow will be treated in section IV. In this section we shall only assume that the mechanism is such that the theory of absolute reaction rates is applicable.

The perpendicular distance between two neighboring aggregates sliding past each other is taken as λ_1 . The average distance between equilibrium positions in the direction of motion is taken as λ , while the distance between neighboring aggregates in this same direction is λ_2 , which may or may not be equal to λ . The distance between aggregates in the plane normal to the direction of motion is written as λ_3 . By definition we have for the viscosity,

$$\eta = f\lambda_1/\Delta v \quad (57)$$

where f is the force per square centimeter tending to displace one layer with respect to the other, and Δv is the difference in velocity of these two layers which are a distance λ_1 apart. Now the process of diffusion is continually taking place, with or without an applied force tending to cause viscous flow. The application of such a force simply tends to make a preferred direction in which the molecules move. On the basis of this picture, the velocity Δv between two successive flow layers is simply the difference between the reaction rate in the direction of flow and in the opposite direction multiplied by the average distance it moves at each reaction. The general equation giving the rate of any reaction which has been modified by some external agency may be written:

$$k' = (kT/h) \exp(-\Delta F^\ddagger/RT - \Delta F^{t'}/RT) \quad (58)$$

Here $\Delta F^{t'}$ is the contribution made by external agency to the free energy of activation, and κ has been set equal to unity. The magnitude of $\Delta F^{t'}$ caused by the applied force may be evaluated in this case as follows: The force acting on a single aggregate is $f\lambda_2\lambda_3$, and it acts to lessen the work of passing over the barrier through the distance $\lambda/2$. That this distance is just $\lambda/2$ follows from the reasonable assumption that the activated complex is a configuration just half way between the initial and final states. Thus the applied force tends to lessen the free energy of activation in the forward direction by an amount $f\lambda_2\lambda_3\lambda/2$, while in the backward direction it is *raised* by the same amount. If the reaction rate constant in the forward direction be denoted by k_f and that in the backward direction by k_b , we have

$$k_f = (kT/h) \exp(-\Delta F^\ddagger/RT + Nf\lambda_2\lambda_3\lambda/2RT) = k \exp(f\lambda_2\lambda_3\lambda/2kT) \quad (59)$$

and

$$k_b = (kT/h) \exp(-\Delta F^\ddagger/RT - Nf\lambda_2\lambda_3\lambda/2RT) = k \exp(f\lambda_2\lambda_3\lambda/2kT) \quad (60)$$

Here k is the specific reaction rate constant giving the number of times per second that an aggregate will jump in the direction of flow when no force is applied, and ΔF^\ddagger is the corresponding free energy of activation.

Now Δv for each aggregate is also Δv for the layer as a whole. Thus the relative rate of displacement of neighboring layers is

$$\Delta v = \lambda k [\exp(f\lambda_2\lambda_3\lambda/2kT) - \exp(-f\lambda_2\lambda_3\lambda/2kT)] \quad (61)$$

or

$$\Delta v = 2\lambda k \sinh (f\lambda_2\lambda_3\lambda/2kT)$$

The viscosity then is given by

$$\eta = f\lambda_1/2\lambda k \sinh (f\lambda_2\lambda_3\lambda/2kT) \quad (62)$$

Since for the forces ordinarily employed in the measurement of viscosity, $f\lambda_2\lambda_3\lambda/2kT \ll 1$, the exponentials in equation 62 may be expanded and higher powers dropped, leaving

$$\eta = kT\lambda_1/\lambda^2\lambda_2\lambda_3k \quad (63)$$

or

$$\eta = (h\lambda_1/\lambda^2\lambda_2\lambda_3) \exp (\Delta F^\ddagger/RT) \quad (64)$$

The formula for the diffusion of one liquid into another when they form perfect solutions is very simply derived as follows: Assume that the concentration gradient is in the X direction and is equal to dC_1/dX and, further, that the distance between two successive potential-energy minima for the diffusing aggregate is λ . Then if the concentration at one minimum is C_1 , that at the next minimum in the positive direction is $(C_1 + \lambda dC_1/dX)$. Now the number of molecules passing through the Y, Z plane per square centimeter in the positive X direction is $N\lambda kC_1$ and in the reverse direction is $Nk\lambda(C_1 + \lambda dC_1/dX)$. The excess proceeding in the negative X direction is $N\lambda^2k dC_1/dX$, which must be equal to $DN dC_1/dX$ from the definition of the diffusion coefficient, D . Thus D is

$$D = \lambda^2k \quad (65)$$

In the case of self-diffusion, k and λ^2 may be eliminated from equations 63 and 65 to give

$$\eta D = kT\lambda_1/\lambda_2\lambda_3 \quad (66)$$

Equation 66 holds for perfect solutions whenever the mechanisms for viscous flow and diffusion are the same. In securing a similar relation for the diffusion of one liquid into another it must be remembered that neither k nor λ^2 is necessarily the same for the diffusing molecule as for the solvent. Designating the molecular species which is acting as the medium by the subscript m and the diffusing species by D , we have

$$\eta D = kT\lambda_1\lambda_D^2k_D/\lambda^2\lambda_2\lambda_3k_m \quad (67)$$

$$\eta D = kT(\lambda_1/\lambda_2\lambda_3)(\lambda_D^2/\lambda_m^2) \exp (\Delta F_m^\ddagger - \Delta F_D^\ddagger) \quad (68)$$

This equation, which applies when the solvent and solute molecules are of very nearly the same size, is to be compared to the well-known Stokes-Einstein diffusion equation,

$$D = kT/6\pi r \quad (69)$$

which is applicable when the diffusing molecule is so large compared to the molecules of the medium in which it is diffusing that by comparison the medium may be thought of as a continuum. Here r is the radius of the diffusing molecule, considered to be spherical. Appropriate variants have been obtained by Stearn and Eyring (61) and by Powell, Roseveare, and Eyring (52) for treating imperfect solutions. If we picture a solute, partitioned at equilibrium between two different solvents, it is clear that

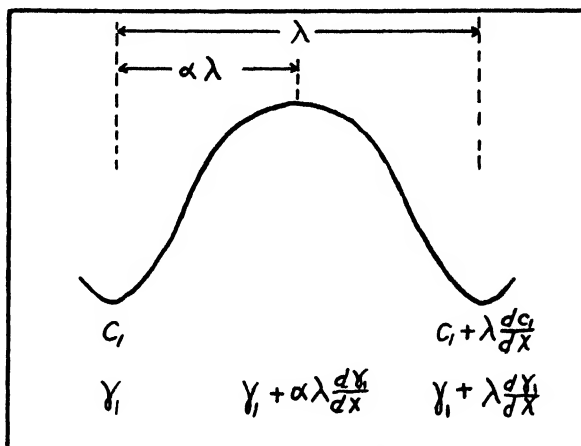


FIG. 4. Potential barrier for diffusion process in non-ideal solution

there is no net diffusion across the interface. Hence it is the activity, and not the concentration, which is the driving force in diffusion.

Consider a unimolecular mechanism by which a diffusing molecule passes over a potential barrier as shown in figure 4, with concentrations and activities as illustrated. The general rate equation (40) will be modified to

$$k = (kT/h) \exp(-\Delta F^\ddagger/RT)(\gamma_n/\gamma^\ddagger) = k^0(\gamma_n/\gamma^\ddagger) \quad (70)$$

where the γ 's are activity coefficients.

The net diffusion rate will then be the difference between the forward and backward rates of transport,

$$\text{Rate}_{\text{net}} = C_1 \lambda k^0 \frac{1}{1 + \frac{\alpha \lambda}{\gamma_1} \frac{d\gamma_1}{dx}} - \left(C_1 + \lambda \frac{dC_1}{dx} \right) \lambda k^0 \frac{1 + \frac{\lambda}{\gamma_1} \frac{d\gamma_1}{dx}}{1 + \frac{\alpha \lambda}{\gamma_1} \frac{d\gamma_1}{dx}} \quad (71)$$

which, upon simplifying, becomes

$$\text{Rate}_{\text{net}} = -\frac{dC_1}{dx} \lambda^2 k^0 \left[1 + \frac{d \ln \gamma_1}{d \ln C_1} \right] \quad (72)$$

Since the diffusion constant is defined by

$$\text{Rate}_{\text{net}} = -D \frac{dC_1}{dx}$$

we have

$$D = \lambda^2 k^0 \left[1 + \frac{d \ln \gamma_1}{d \ln C_1} \right] \quad (73)$$

If the molal volumes of the solute and solvent molecules, V_1 and V_2 , are not too different, equation 73 may be put in the form

$$D = \lambda^2 k^0 \frac{d \ln a_1}{d \ln N_1} \quad (74)$$

In writing equation 74 for equation 73, a small correction factor,

$$1 - N_1 \left(1 - \frac{V_1}{V_2} \right)$$

due to changing from concentration units to mole fraction, is neglected. This factor reduces to unity for $V_1 = V_2$, and even, in general, since it is a linear function of mole fraction, it will simply be absorbed into the corresponding values of λ^2 . For systems such that ΔF^\ddagger for viscous flow is the same as ΔF^\ddagger for diffusion, we would have

$$\eta D = \frac{\lambda_1}{\lambda_2 \lambda_3} kT \frac{d \ln a_1}{d \ln N_1} \quad (75)$$

IV. THE FREE ENERGY OF ACTIVATION FOR VISCOUS FLOW

In this section an attempt will be made to identify the quantities occurring in equation 64 with properties characteristic of the liquid to which it is being applied. To do this we shall employ the theorem, "The amount of energy required to make a hole in the liquid the size of a molecule is equal to the energy of vaporization" (19). This theorem is easily derived as follows: Suppose we have N molecules forming a liquid. Then each of them is bound to the others by bonds adding up to the total energy

$$E = \sum_i n_i E_i$$

where n_i is the number of bonds of a particular kind, each of which has the bond strength E_i . To vaporize the N molecules requires an energy

of $NE/2$, since each bond belongs to two molecules. Therefore to vaporize a single molecule requires the energy $E/2$, providing no hole is left in the liquid. However, if we vaporize one molecule leaving the hole, we must supply exactly the energy E . If we then return this gas molecule to the liquid we get back the energy $E/2$, so that it requires rigorously the same energy $E/2$ to make a hole in a liquid of a size which will just accommodate a single molecule as it does to vaporize one molecule without leaving a hole. Clearly a large hole will require more energy for its formation than a small one, but the energy of formation of a hole need not, and in general will not, be strictly proportional to the size of the hole. This is illustrated by the effect of pressure on viscosity discussed in a later section.

In relating the quantities occurring in equation 64 with properties of the liquid, the first thing to decide is whether the flow aggregate is a single molecule or a group of molecules. In making the decision as to which is the more probable mechanism, a guiding principle that applies to all rate processes must be kept in mind: *viz.*, any rate process proceeds by all possible mechanisms and therefore chiefly by the fastest ones. If different possible mechanisms do not involve greatly varying values of the contribution of the entropy of activation to the free energy of activation, then the fastest process will be the one with the smallest energy of activation. Now a molecule can flow only if there is a hole in the liquid for it to flow into, and thus the difference in energy between the activated and normal states is chiefly due to the extra volume required by the activated state. Hence the most probable mechanism is one that requires the smallest amount of extra volume for the process to take place. Clearly, this condition will be most nearly satisfied by a mechanism involving only one or two molecules. Figure 5 illustrates one suggested mechanism by means of which one molecule could move past another. The figure illustrates the instantaneous formation of a double molecule which then rotates through an arc of about 90° . The initial position of the molecules is illustrated by the dotted lines, and after rotation they occupy the position indicated by the heavy black circles.

This mechanism identifies the λ 's of the flow aggregate with the dimensions of a single molecule. If, further, the distance between successive minima, λ , is identified with λ_1 , the perpendicular distance between adjacent layers of molecules, then the product $\lambda_1\lambda_2\lambda_3$, in the denominator of equation 64, is just equal to V/N , where V is the molal volume of the liquid.

Equation 64 may then be written,

$$\eta = (\hbar N/V) \exp(\Delta F^\ddagger/RT) \quad (76)$$

There remains the problem of deciding the method for determining the free energy of activation for viscous flow. This may be estimated as some fraction of the free-energy change of an analogous reaction. Powell, Roseveare, and Eyring (52) base their choice of the analogous reaction on the following model of the unit flow process: An individual molecule occasionally acquires the activation energy necessary to squeeze past its

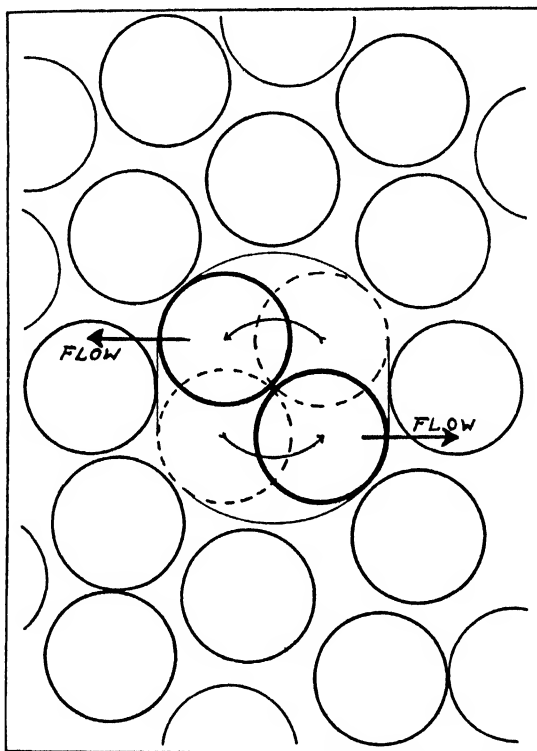


FIG. 5. Viscous flow by means of double molecules as illustrated by Hirschfelder, Stevenson, and Eyring (30). Two molecules collide to form a double molecule. If there is sufficient space available, this double molecule can rotate and then dissociate. One layer of liquid can flow past another by a succession of these processes.

neighbors into the next equilibrium position. The bonds which must be broken are the same bonds that would be broken in the process of vaporization. However, the work of expansion to the vapor state will not be needed, and the entropy of this expansion will not be available. Thus we may expect ΔF^\ddagger to be correlated with

$$\Delta F_{\text{vap}} + T\Delta S_{\text{vap}} - RT = \Delta H_{\text{vap}} - RT = \Delta E_{\text{vap}}$$

In figure 6, ΔF^\ddagger for viscous flow has been plotted against ΔE_{vap} for ninety-three inorganic and organic liquids at their boiling points. The points fall along a straight line which passes through the origin, and has a slope 1/2.45. Equation 76 can then be written

$$\eta = Nh/V \exp(\Delta E_{\text{vap}}/2.45RT) \quad (77)$$

When ΔH^\ddagger is plotted against ΔE_{vap} , the results for normal liquids are about as good as for ΔF^\ddagger , but hydrogen-bonded liquids involve large deviations in the direction of high activation energies. These abnormal activation energies are in large measure compensated by abnormal entropies of

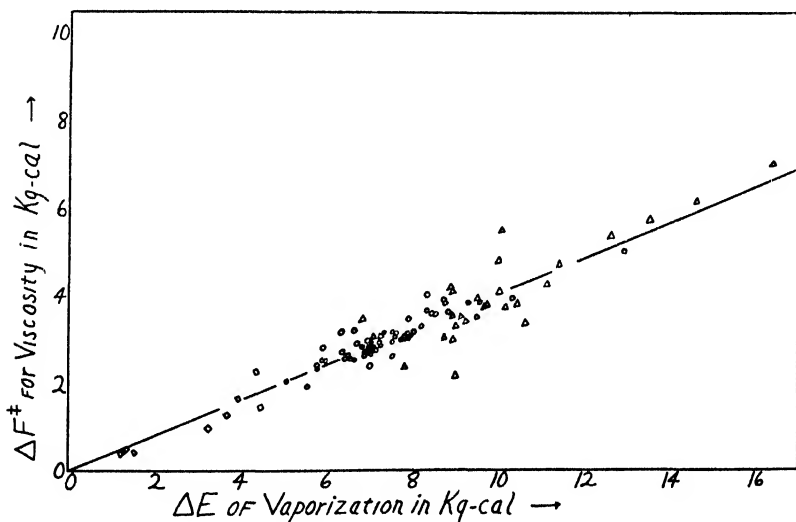


Fig. 6. The squares represent "permanent gases," the triangles represent hydrogen-bonded liquids, and the circles represent the other liquids.

activation. This compensation is a common phenomenon for both rate and equilibrium processes.

It is of interest to examine other proposed equations which, in general, permit prediction of viscosity to within a factor of 2 to 3. We first recall that ΔF^\ddagger includes one more degree of freedom for the normal than for the activated state. The degree of freedom corresponding to decomposition of the activated complex is omitted from ΔF^\ddagger , being included in the frequency kT/h . If classical statistics is applicable, there is no difference in the parts of the partition functions depending on kinetic energy for the normal and for the activated state. This may be seen by inspection of equations 25 and 26 if it is recalled that in classical mechanics the kinetic

energy, T' , is independent of the potential field to which the system is confined. If we then make the approximation that the partition function for the liquid as a whole is obtainable from the partition function for an average molecule moving in the field of its neighbors, we have:

$$f = (2\pi mkT/h^2)^{3/2} \iiint \exp(-V/RT) dx dy dz \quad (78)$$

$$= (2\pi mkT/h^2)^{3/2} v_f$$

where V is the potential due to the presence of neighbors and

$$v_f \left(\equiv \iiint \exp(-V/RT) dx dy dz \right)$$

is the free volume. Only translational degrees of freedom are considered in equation 78. Since the extra degree of freedom in the normal molecules is a translation, we have, from equations 76 and 78,

$$\eta = (hN_A/V)(2\pi m^*kT/h^2)^{1/2} v_f^{1/3} \exp(\Delta F'/RT) \quad (79)$$

Here $\Delta F'$ includes all contributions to the free energy of activation for viscous flow other than those involving the degree of freedom in which decomposition is occurring.

Equation 79 contains two unknown quantities, v_f and $\Delta F'$. The free volume plays an important rôle in many properties of liquids other than the viscosity, and a discussion of its evaluation is given below. $\Delta F'$ may be divided into the energy of activation for viscous flow, ΔE_{vis} , and an entropy of activation. Since a molecule cannot flow unless there exists a cavity for it to flow into, ΔE_{vis} is due chiefly to the energy of forming a hole in the liquid. It is thus closely related to the energy of vaporization, a relationship which has already been discussed. By comparison with experiment it is found that the entropy term in $\Delta F'$ is small and may to a good approximation be set equal to zero.

V. FREE VOLUMES OF MOLECULES IN LIQUIDS

A. Free volumes from the energy-volume coefficient

In order to obtain values of the free volume, v_f , to use in equation 79, it is necessary to consider certain simple models for liquids. One simple model that has been used is that employed by Eyring and Hirschfelder (21). It is assumed that the potential energy does not change as the molecule moves from its equilibrium position until it collides with its neighbors. At this point the potential energy goes to infinity. The molecule thus moves in a potential box with a flat bottom and straight sides, the size of the box being governed by the total volume and the size and packing of

the molecules. If, further, the rotational motion of the molecules is not changed on changing the volume of the liquid or on vaporization, a term for it need not be included in the partition function for the liquid in considering the vapor pressure or the equation of state. The same thing may be said for the degrees of freedom corresponding to internal vibrations.

The mathematical statement of this picture is as follows: If f^N is the partition function for the liquid, it may be written

$$f^N = [(2\pi mkt/h^2)^{3/2}(v_f)]^N \exp(\Delta S_c/R) \exp(-\Delta E_{vap}/RT) \quad (80)$$

where ΔS_c would be equal to zero if the function f^N were like that for a solid and would equal R if it were like that for a gas. Hirschfelder, Stevenson, and Eyring (30) considered that ΔS_c should approach the limiting value R and called it communal entropy. Lennard-Jones and Devenshire (40) and Monro and Kirkwood (47) have more recently estimated this extra randomness of liquids over that for solids. Lennard-Jones and Devenshire assumed that it arose from a random distribution between lattice positions present in the solid and new lattice positions which accompanied the increase in volume during melting and subsequently. Later in this paper we shall have more to say about the nature of ΔS_c . To the approximation employed here, the energy of vaporization, ΔE_{vap} , is just the difference in potential energy between the liquid and gas. This follows because the kinetic-energy terms are the same for both phases, and it is assumed that no potential-energy terms are associated with either the rotation of the molecules or with their oscillation about their equilibrium positions. v_f is the size of the box in which the geometrical center of the molecule can move without change in potential energy.

In order to determine v_f , suppose that the molecules in the liquid are arranged in a simple cubic lattice and that each molecule, on the average, can move until it touches its neighbors when they are in their mean positions. Then if d is the incompressible diameter of each molecule, we have from figure 7 that $2(V/N)^{1/3} - 2d$ is the distance that the central molecule is free to move along each axis. v_f is then this quantity cubed,⁶ i.e.,

$$v_f = 8[(V/N)^{1/3} - d]^3 \quad (81)$$

The equation of state is obtained by differentiation of $\ln f^N$ with respect to the volume and multiplication by kT .

We have from equations 13, 18, and 80,

$$P = -kT(\partial \ln f^N / \partial V)_T = RT(\partial \ln v_f / \partial V)_T - (\partial \Delta E_{vap} / \partial V)_T \quad (82)$$

⁶ It will sometimes be convenient to use the quantity $Nv_f = V_f$. Similarly, we shall use $v = V/N$ to indicate the volume per molecule.

From equation 81

$$(\partial \ln v_f / \partial V)_\tau = (V - N^{1/3} dV^{2/3})^{-1} = 2/V^{2/3} V_f^{1/3} \quad (83)$$

On combining equations 82 and 83 we arrive at

$$[P + (\partial \Delta E_{vap} / \partial V)_\tau] V^{2/3} V_f^{1/3} = 2RT \quad (84)$$

an equation which relates the free volume to known properties of the liquid. In employing equation 84 it must be remembered that the quantity $(\partial \Delta E_{vap} / \partial V)_\tau$ is, in general, not known and must be estimated. A closely related quantity is $(\partial E / \partial V)_\tau$, but this is also frequently unknown. The difficulty is resolved with the aid of the generalization, pointed out by Hildebrand (28), that $(\partial E / \partial V)_\tau$ is very nearly equal to $\Delta E_{vap} / V$ at low pressures for a large number of cases. Water and other hydrogen-bonded liquids, liquid metals, and all liquids at high pressures have values of $(\partial E / \partial V)_\tau$ which are less than $\Delta E_{vap} / V$. Free volumes computed from

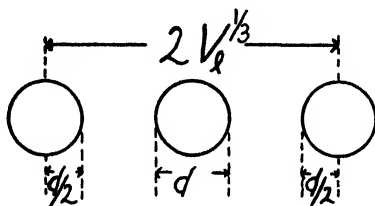


Fig. 7. The relationship between the free and the total volume

$$v_f^{1/3} = 2v^{1/3} - 2d$$

equation 84 agree well with those obtained by other methods, which are discussed below. Further considerations similar to those discussed above lead to reasonable agreement with experiment for expansion coefficients and compressibilities of liquids, as well as to a derivation of Trouton's and Hildebrand's rules (21).

B. Free volumes from velocity of sound

It has been found possible to modify certain kinetic theory formulae for gases in such a way as to make them applicable to liquids (37). The point of view employed is to treat the molecule moving in its free volume in the liquid as equivalent to the molecule moving in the total volume in the gas phase.

The velocity of sound, u , in any homogeneous medium is given by the general hydrodynamic formula:

$$u = (V/\beta_s)^{1/2} \quad (85)$$

where v is the specific volume and β_s is the adiabatic compressibility defined by

$$\beta_s \equiv -(1/V)(\partial V/\partial P)_s$$

For the special case of an ideal gas, equation 85 becomes

$$u = (RT\gamma/M)^{1/2} \quad (86)$$

where γ is the ratio of the specific heat at constant pressure to that at constant volume, and M is the molecular weight of the gas.

Equation 86 may be compared to that for \bar{c} , the average kinetic theory velocity of the molecules.

$$\bar{c} = (8RT/\pi M)^{1/2} \quad (87)$$

It is seen that u is proportional to \bar{c} but is slightly smaller, since the factor $(8/\pi)^{1/2}$ is always greater than $\gamma^{1/2}$. This is what one might suppose, since a wave propagated by matter would hardly be expected to travel

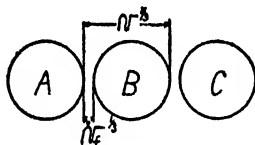


FIG. 8. Illustration of the mechanism which explains the observation that the velocity of sound in liquids is greater than the kinetic theory velocity of the molecules.

faster than the molecules which carry it. However, when the velocity of sound in liquids is compared to the kinetic theory velocity of the molecules, it is found that u for most liquids is greater than \bar{c} by factors ranging from 5 to 10. For example, for benzene at 25°C., \bar{c} is 2.83×10^4 , u (gas) is 1.88×10^4 , while u (liquid) is 13.0×10^4 cm. per second. Figure 8 illustrates the mechanism responsible for the fact that the velocity of sound in liquids can be so much greater than the kinetic theory velocity of the molecules. The wave front is assumed to travel from the edge of molecule A to the adjacent edge of molecule B with the velocity given by equation 86. As A collides with B, however, the signal is transmitted almost instantaneously to the opposite edge of molecule B.

This follows from the fact that sound waves are longitudinal, or compression, waves. Since the ratio of the total distance to the free space between two points in the liquid is given by the ratio $(V/V_f)^{1/3}$, this leads at once to the equation,

$$u \text{ (liquid)} = u \text{ (gas)} (V/V_f)^{1/3} = (RT\gamma/M)^{1/2} (V/V_f)^{1/3} \quad (88)$$

Equation 88 may be employed either to find the velocity of sound in liquids if V_f is known from some other source, or to determine free volumes if sound velocity measurements have been made.

C. The relation between free volume and thermal conductivity in liquids

A test of the general point of view outlined above is desirable, and is provided by considering the thermal conductivity of liquids (37).

A straightforward application of the kinetic theory of gases gives the relation for the thermal conductivity K ,

$$K(\text{gas}) = (1/3)(N/V)\bar{c}Lc_v \quad (89)$$

where N/V is the number of molecules per cubic centimeter, L is the mean free path, and c_v is the specific heat per molecule. It is found, however, that equation 89 gives values which are too low for the thermal conductivity of gases, and Eucken (11) gives the correction factor $1/4(9\gamma' - 5)$, where γ' is the ratio of c_p to c_v . Equation 89 may now be rewritten,

$$K(\text{gas}) = (1/3)[1/4(9\gamma' - 5)](N/V)\bar{c}Lc_v \quad (90)$$

a relation which may be tested in the form,

$$K(\text{gas}) = \eta(c_v/m)(1/4)(9\gamma' - 5) \quad (91)$$

since the viscosity, η , is given by $(1/3)(N/V)m\bar{c}L$. Equation 91 has been observed to give excellent agreement with experiment (42).

In order to convert equation 90 into a form applicable to liquids the following identifications may be made: (N/V) becomes the number of molecules per cubic centimeter of liquid rather than per cubic centimeter of gas. The average velocity \bar{c} must be multiplied by the ratio $(V/V_f)^{1/3}$, for the same reasons as are given above for justifying equation 88. The distance that the energy is carried is now $(v)^{1/3}$ rather than L , the mean free path for the gas. c_v for the gas is replaced by αc_v , where α is the accommodation coefficient which takes account of the number of degrees of freedom which come into equilibrium in the thermal conduction process. Making all these substitutions we have:

$$K(\text{liquid}) = (1/12)(9\gamma' - 5)(1/v)(8RT/\pi M)^{1/2}(V/V_f)^{1/3}(v)^{1/3}(\alpha c_v) \quad (92)$$

On substituting equation 88 and simplifying, we have

$$K(\text{liquid}) = (1/12)(9\gamma' - 5)(8/\pi\gamma)^{1/2}(N/V)^{2/3}u(\alpha c_v) \quad (93)$$

Here u is the velocity of sound in the liquid, γ' is the effective value of the ratio of c_p to c_v for thermal conduction in the gas, and γ is the same quantity for the transmission of sound.

Equation 93 is very similar to one given by Bridgman for the thermal

conductivity of liquids (5, 6). In order to have equation 93 reduce to the one successfully employed by Bridgman, αc_v must be assigned the value $3k$ per molecule. For the liquids composed of polyatomic molecules, which Bridgman studied, this is understandable if only the kinetic energy of translation and rotation transfer appreciable energy in the process of thermal conduction. Using a value of αc_v equal to $3k$, γ' becomes $4/3$ and equation 93 reduces to

$$K(\text{liquid}) = (0.931/\gamma^{1/2}) 3k (N/V)^{2/3} u \quad (94)$$

This formula has the same form as that employed by Bridgman, but the numerical coefficient is about 12 per cent smaller, as a result of the factor $0.931/\gamma^{1/2}$ replacing unity in his expression.

TABLE 1

Comparison of the observed thermal conductivities of liquids at 30°C. with those computed from equation 94

SUBSTANCE	K (OBSERVED (5))	K (CALCULATED)	$\frac{K(\text{OBSERVED})}{K(\text{CALCULATED})}$
	$\times 10^8$ C.G.S. units	$\times 10^8$ C.G.S. units	
Methyl alcohol	21.1	22.3	0.95
Ethyl alcohol.	18.0	19.2	0.94
Propyl alcohol	15.4	17.3	0.89
Butyl alcohol	16.7	13.3	1.26
Isoamyl alcohol	14.8	14.1	1.05
Ether	13.7	10.9	1.26
Acetone	17.9	16.3	1.10
Carbon disulfide	15.9	16.1	0.99
Ethyl bromide	12.0	12.8	0.94
Ethyl iodide	11.1	10.6	1.05
Water	60.1	51.7	1.16

Table 1 shows values for K at 30°C. for a number of liquids computed by equation 94 for comparison with Bridgman's experimental values. The agreement, somewhat better than Bridgman's earlier computations, suggests that the general point of view cannot be far wrong. Equation 94 not only works remarkably well for the variation of K from liquid to liquid at atmospheric pressure, but, as Bridgman has pointed out, it also gives approximately the temperature variation of K . It is, therefore, quite surprising that it does not predict the pressure effect correctly. Whereas most liquids increase their thermal conductivity by approximately a factor of 2 on going from atmospheric pressure to 12,000 atmospheres, the formula predicts an increase by about a factor of 4. It has been suggested that such a decrease in the value of α might be due to quantization of the

mass motions of the molecules at high pressures (37). The effective heat capacity, α_{ν} , would have to become $1.5k$ in order to explain the observations at the highest pressures to which the experiments extend.

D. A comparison of values of free volumes of liquids computed by different methods

Table 2 gives a comparison of free volumes for a number of liquids calculated from equations 84 and 88. The agreement indicates that, for the type of liquid considered in table 2 at atmospheric pressure, it is immaterial whether equation 84 or equation 88 is employed in the viscosity equation. Since equation 84 fails badly when applied to liquid mercury (21), it provides no check of the sound velocity equation 88 for this case. Consequently it is of interest to compare equation 88 with an equa-

TABLE 2
Values of the free volume for various liquids (30)

Sub- stance	Acetone		Ether		Chloroform		Toluene		Carbon tetrachloride		Carbon disulfide	
	V_f FROM EQUATION 88	V_f FROM EQUATION 84	V_f FROM EQUATION 88	V_f FROM EQUATION 84	V_f FROM EQUATION 88	V_f FROM EQUATION 84	V_f FROM EQUATION 88	V_f FROM EQUATION 84	V_f FROM EQUATION 88	V_f FROM EQUATION 84	V_f FROM EQUATION 88	V_f FROM EQUATION 84
°C.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.	cc.
0			0.48	0.47	0.21	0.25	0.16	0.16	0.16	0.21	0.23	0.45
10			0.57	0.55	0.24	0.29	0.19	0.19	0.24	0.26	0.26	0.52
20	0.45	0.54	0.70	0.65	0.29	0.34	0.22	0.22	0.28	0.31	0.30	0.58
30			0.90	0.79	0.34	0.40	0.26	0.26	0.33	0.37	0.36	0.66
40	0.64	0.63			0.40	0.47	0.31	0.31	0.38	0.43	0.39	0.75
50					0.48		0.36	0.36	0.45	0.50	0.47	0.84

tion for the free volume of mercury which is known to be consistent with its thermodynamic properties. The simplest model which might be employed is to assume that the Einstein characteristic temperature is the same for the liquid as for the solid phase. Application of equation 78 should then lead to approximate values for v_f . A better value of the characteristic temperature of the liquid may be obtained by choosing it so as to fit the observed entropy of fusion (36). Further, since the observed values for the specific heat at constant volume for mercury fall below the classical value for a harmonic oscillator as the temperature is raised, the expression for the free volume must be modified to take account of this effect. An expression for V_f consistent with these requirements is

$$V_f = N \left[\frac{T/\Theta}{(2\pi mkT/h^2)^{1/2}} + 2(V/N)^{1/3} - (2V_0/N)^{1/3} \right]^3 \quad (95)$$

Here Θ is the characteristic temperature of the liquid and V_0 is identified as the volume of the liquid at the melting point. The form of the potential function corresponding to equation 95 is shown in figure 9, and values for V_f computed from equation 95 are compared with those obtained from the sound velocity equation in table 3. The general agreement is of particular

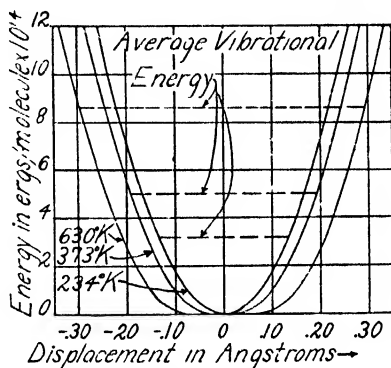


FIG. 9. Potential-energy function for displacements of a mercury atom from its equilibrium position (37).

TABLE 3

Free volumes for liquid mercury at different temperatures and pressures

t	P	V_f FROM EQUATION 88	V_f FROM EQUATION 95
$^{\circ}\text{C.}$	atmospheres	cc.	cc.
-39	1		0.0081
0	1	0.0120	0.0120
100	1	0.0220	0.0256
200	1		0.0434
300	1		0.0718
357	1		0.0904
0	1	0.0120	0.0120
0	1000	0.0117	0.0116
0	2000	0.0114	0.0114
0	3000	0.0111	0.0111
0	4000	0.0107	0.0109
0	5000	0.0104	0.0107
0	6000	0.0101	0.0105
0	7000	0.0098	0.0103

interest, since the free volumes are of a different order of magnitude than those of the non-metallic liquids discussed above.

VI. THE VISCOSITY OF LIQUIDS

Since means are now available for estimating the free volume, it is possible to apply equation 79 to the problem of the viscosity of liquids.

The equation may be rewritten in the form,

$$\eta = (hN/V)(2\pi m^*kT/h^2)^{1/2} v_f^{1/3} \exp(\Delta E_{vis}/RT) \exp(-\Delta S'/R) \quad (96)$$

where $\Delta E'$ will be some fraction of the energy of vaporization. This follows from the assumption that the energy of activation for viscous flow is due chiefly to the extra volume required by the activated complex, since the energy of formation of a hole of molecular size is equal to the energy of vaporization. The hole required for the flow process to take place will not, in general, be as large as a cavity of molecular dimensions, since this would be unnaturally extravagant of free energy. The bimolecular mechanism illustrated in figure 5, for example, would require a cavity of about one-third the size of a molecule. The situation is somewhat different when each molecule forms directed bonds (e.g., hydrogen bonds) with its neighbors, and these cases will be discussed later.

For those cases where $(\partial \Delta E_{vap}/\partial V)_T$ may be taken equal to $\Delta E_{vap}/V$, and where the external pressure may be neglected in comparison to $\Delta E_{vap}/V$, equation 84 reduces to

$$V_f^{1/3} = 2RTV^{1/3}/\Delta E_{vap} \quad (97)$$

If, further, ΔE_{vis} is written as $\Delta E_{vap}/n$, then equation 96 may be written

$$\eta = 7.71 \times 10^{-4} (M^{1/2} T^{2/3} / V^{2/3} \Delta E_{vap}) \exp(\Delta E_{vap}/nRT) \exp(-\Delta S'/R) \quad (98)$$

where ΔE_{vap} is in calories per mole.⁷ Equation 98 has been tested by Ewell and Eyring (16) in the following manner: The n appearing in the exponential of equation 98 was chosen to give the correct temperature coefficient of viscosity by computing values for η neglecting the entropy of activation. The computed values of the viscosity are then plotted as $\ln \eta$ against $1/T$. The value of n which gives a plot parallel to the straight line of the observed viscosities is taken as the value of n which gives the correct temperature coefficient. The calculations were carried out for most of the liquids for which reliable values of the viscosities and the heats of vaporization are known over a temperature range. The results to the nearest half integer are given in table 4. As examples, table 5 shows the calculations for carbon tetrachloride, and figure 10 shows the plots for carbon tetrachloride, nitrogen, hexane, and chloroform. Computations similar to those

⁷ The coefficient 7.71×10^{-4} given in equation 98 is smaller by a factor equal to $\sqrt{2}$ than the coefficient given by Ewell and Eyring (16). This is because Ewell and Eyring assumed a unimolecular mechanism, while a bimolecular mechanism is assumed in equation 98. It is difficult to choose between the unimolecular and bimolecular mechanism from the viscosity data alone, but when this is taken in conjunction with diffusion data, the unimolecular mechanism seems the more likely, as we shall see.

THEORY OF ABSOLUTE REACTION RATES

given in table 5 and plots similar to those in figure 10 were made for all the liquids listed in table 4.

Inspection of table 5 reveals the fact that there seems to be some correlation between the symmetry and polarity of the molecule and the value of n required to give the correct temperature coefficient of viscosity. Thus, all the liquids with a value of n equal to 3 are non-polar,⁸ and many of them are spherically symmetrical. This is certainly true of carbon tetrachloride, methane, and argon and is approximately true of nitrogen and carbon monoxide when the kinetic theory shell is considered. The liquids

TABLE 4
Values of n for different substances

$n = 3$	Carbon tetrachloride, benzene, cyclohexane, methane, nitrogen, carbon monoxide, argon
$n = 3.5$	Dichloroethane, dibromoethane, oxygen
$n = 4$	Pentane, hexane, heptane, carbon disulfide, chloroform, toluene, ether, ethyl acetate, acetone, ethyl iodide, ethyl bromide, methyl iodide, ethylene

TABLE 5
Computation of viscosity of carbon tetrachloride

t	V	ΔE_{vap}	η (OBSERVED)	η (CALCULATED) + η (OBSERVED)		
				$n = 2$	$n = 3$	$n = 4$
$^{\circ}\text{C.}$	<i>cc per mole</i>	<i>kg.-cal. per mole</i>	<i>millipoises</i>			
0	94.3	7.56	13.47	30.7	2.12	0.95
20	96.6	7.30	9.69	24.0	2.11	1.04
40	99.0	7.06	7.38	19.7	2.07	1.13
60	101.6	6.81	5.84	16.4	2.06	1.24
80	104.4	6.56	4.68	14.2	2.08	1.36

with values of n greater than 3 are not spherically symmetrical and most of them are polar. If the molecule is not symmetrical and makes a better bond with one neighbor than with the others, it will be able to preserve this bond in the activated state, so that the energy of activation will tend to fall *below* the normal value for symmetrical molecules. On the other hand, if a molecule forms strong directional bonds with a number of nearest neighbors, the energy of activation will tend to be *above* the normal value. Molecules with a single large dipole, such as ethyl iodide, ethyl

⁸ Carbon monoxide has a small dipole moment of the order of 0.15 Debye unit.

bromide, and acetone, are examples falling in the first category, while water (to be discussed later) exemplifies the second.

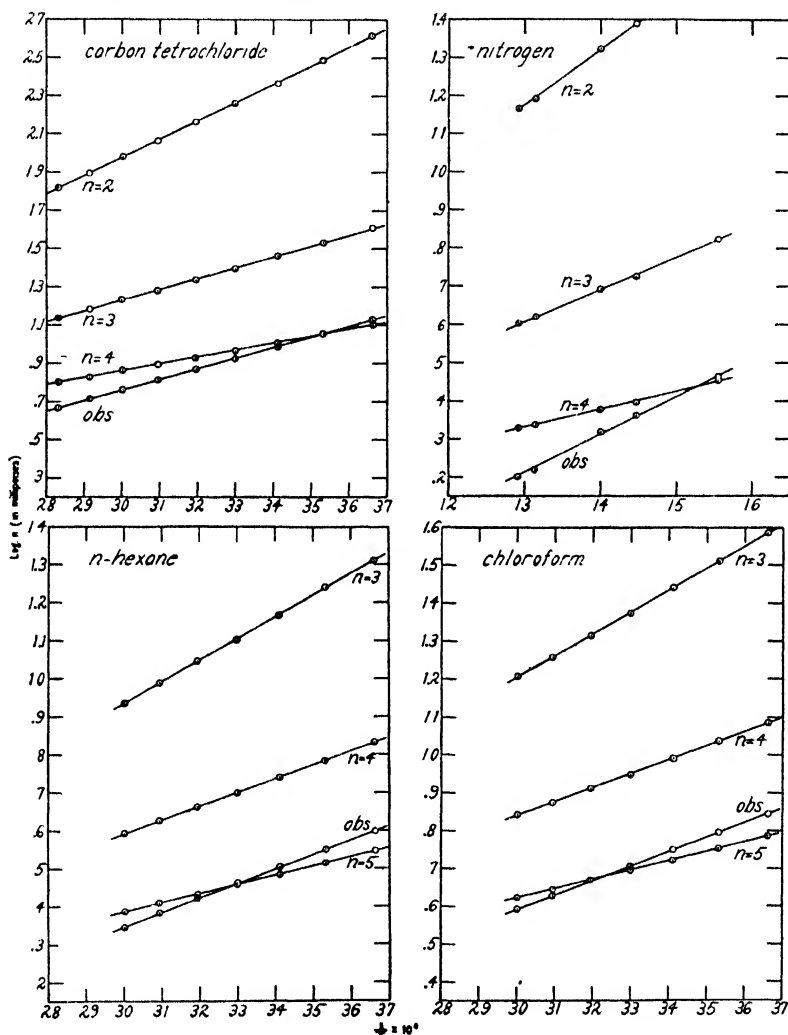


FIG. 10. Plots of $\log \eta$ versus $1/T$ for observed and calculated viscosities, using integral values of n in equation 98 (from Ewell and Eyring (16)). Ordinates are $\log \eta$ in millipoises; abscissas are $\frac{1}{T} \times 10^4$.

For the rolling mechanism indicated in figure 5, the ratio of the size of the hole required to the molecular volume will vary with the liquid struc-

ture, which in turn will depend on the shape of the molecules. Experiments seem to indicate that for the long cylinders, such as the paraffins, this ratio is smaller (about 1:4) than for spherical molecules, where the ratio is about 1:3. A different value for n is to be anticipated for molecules with shapes which preclude the above mechanism. Large flat molecules of the anthracene type will presumably provide such examples.

TABLE 6
Comparison of values of n with $\Delta E_{\text{vap}}/B$

LIQUID	B <i>kg.-cal. per mole</i>	ΔE_{vap} AT	$\frac{\Delta E_{\text{vap}}}{B}$	n
		BOILING POINT <i>kg.-cal. per mole</i>		
CCl_4	2.50	6.60	2.7	3
C_2H_6	2.54	6.66	2.6	3
C_6H_{12} (cyclohexane)	2.89	6.70	2.3	3
CH_4	0.72	1.82	2.5	3
A	0.52	1.42	2.8	3
N_2	0.45	1.21	2.7	3
CO	0.47	1.31	2.8	3
O_2	0.40	1.47	3.7	3.5
$\text{C}_2\text{H}_4\text{Cl}_2$	2.27	6.93	3.1	3.5
$\text{C}_2\text{H}_4\text{Br}_2$	2.59	7.89	3.0	3.5
C_5H_{12} (pentane)	1.58	5.51	3.5	4
C_6H_{14} (hexane)	1.72	6.22	3.6	4
CHCl_3	1.76	6.63	3.8	4
$\text{C}_2\text{H}_5\text{I}$	1.72	6.40	3.7	4
$\text{C}_2\text{H}_5\text{Br}$	1.59	6.08	3.8	4
CS_2	1.28	5.92	4.6	4
$\text{C}_6\text{H}_5\text{CH}_3$	2.12	7.24	3.4	4
$(\text{C}_2\text{H}_5)_2\text{O}$	1.61	5.70	3.5	4
CH_3COCH_3	1.66	6.40	3.9	4
C_2H_4	0.79	3.50	4.4	4

Inspection of figure 10 shows that the plots of the logarithms of the viscosity against the reciprocal of the temperature are straight lines for both the calculated and the observed curves. This means that they can be fitted with an equation of the form

$$\eta = A \exp (B/RT) \quad (99)$$

Here A is an entropy-dependent factor, and B is an energy factor.

Then B will be the experimentally determined quantity, $R d \ln \eta / d(1/T)$. Because this can be taken as a constant for most liquids throughout their

normal liquid range, it can be considered to be an average activation energy for viscous flow. Since ΔE_{vap} varies only slowly with temperature, the ratio $\Delta E_{\text{vap}}/B$ should be approximately equal to 3 or 4 anywhere in the temperature range where B is a constant. Table 6 gives a comparison of values of n , as determined by the method given above, with $\Delta E_{\text{vap}}/B$, where ΔE_{vap} is taken at the normal boiling point for all the liquids in the table. Inspection of table 6 shows that there is a correlation between the values of n and the ratio $\Delta E_{\text{vap}}/B$, so that this ratio may be taken as a rough measure of n .

A correlation between B and ΔE_{vis} having been established, it is of interest to examine further approximate values of n for quite unsymmetrical molecules, cases for which complete data for applying equation 98 are not available. Table 7 shows values of $\Delta E_{\text{vap}}/B$ for the normal paraffin hydro-

TABLE 7
Values of $\Delta E_{\text{vap}}/B$ for normal paraffin hydrocarbons

HYDROCARBON	RANGE °C.	B	ΔE_{vap}	$\Delta E_{\text{vap}}/B$
		kg-cal per mole	kg-cal per mole	
$n\text{-C}_5\text{H}_{12}$	0 b.p.	1.58	5.71	3.6
$n\text{-C}_6\text{H}_{14}$	0-b.p.	1.73	6.96	4.0
$n\text{-C}_7\text{H}_{16}$	0-b.p.	1.91	8.11	4.3
$n\text{-C}_8\text{H}_{18}$	0-b.p.	2.14	9.21	4.3
$n\text{-C}_9\text{H}_{20}$	0-40	2.41	10.21	4.2
$n\text{-C}_{10}\text{H}_{22}$	0-30	2.60	11.11	4.3
$n\text{-C}_{11}\text{H}_{24}$	0-30	3.06	11.96	3.9
$n\text{-C}_{14}\text{H}_{30}$	20-40	3.60	14.21	4.0
$n\text{-C}_{16}\text{H}_{34}$	20-40	4.01	15.51	3.9
$n\text{-C}_{18}\text{H}_{38}$	40-60	4.15	16.76	4.0

carbons (16). This ratio is seen to be about 4 for all these molecules. Part of the increase in the ratio with increasing molecular weight is due to the fact that the comparison is made at 25°C. rather than at corresponding temperatures, such as their boiling points. Here, again, the energy of activation for viscous flow is seen to be about one-fourth the energy of vaporization. This result for long-chain molecules is consistent with the mechanism illustrated in figure 5, if the circles in figure 5 illustrate cylinders rolling over each other as viewed along their axes. A pile of logs might be expected to roll over each other by an analogous mechanism.

A. The entropy of activation for viscous flow

Inspection of figure 10 will reveal that the plots of the observed viscosity and the parallel ones which were calculated neglecting $\Delta S'$ do not coincide. The calculated values are greater by an average factor of about 2, when

the value of n giving the right temperature coefficient is used and the entropy of activation is neglected. The following are the factors by which the viscosities calculated, neglecting the factor $(-\Delta S'/R)$, are too high: carbon tetrachloride, 2.5; benzene, 2.5; cyclohexane, 1.5; methane, 2.5; argon, 1.6; nitrogen, 1.7; carbon monoxide, 1.8; pentane, 1.2; hexane, 1.2; heptane, 1.4; carbon disulfide, 1.3; ether, 1.4; ethyl acetate, 1.5; toluene, 2.5; acetone, 1.6.

If all the other terms in the equation are correctly evaluated, these factors indicate a value of $\Delta S'$ of the order of one entropy unit. That the activated configuration should have a greater entropy than the normal one is a reasonable result. Indeed, it would be surprising if $\Delta S'$ were exactly zero, and it is an interesting fact that it is almost equal for such a wide variety of substances.

A number of the approximations that have been made in order to apply equation 98 may be responsible for part of this factor which is interpreted as $\Delta S'$. The expression 97 for $V_f^{1/3}$ may not be just equal to what one would obtain by an exact integration over potential energy if such an integration could be carried out. κ may not be unity for the flow process, and a value smaller than unity would lead to greater calculated entropies of activation. The identification of λ and λ_1 may not be justified in all cases.

We now consider in greater detail the methods available for estimating the energy of activation. Various thermodynamic processes may be used to estimate the energy of forming a hole. How exact an estimate they provide of the energy of activation for viscous flow depends on how nearly the thermodynamic process approximates the formation of the hole in the flow process. The energy of vaporization measures exactly the cost in energy of a hole into which a molecule fitted if *all the other molecules remained exactly as they were before the molecule was removed*. However, the molecules surrounding such a hole will tend to decrease this free energy somewhat by reorienting. This effect has been considered in detail by Kirkwood (39).

The thermodynamic quantity $(\partial E/\partial V)_T$ measures the energy required for a uniform expansion of the liquid. This function, multiplied by the extra volume required by the activated complex, gives the energy of activation for viscous flow *if the uniform volume expansion takes place in just the same way as the process of forming a hole required for viscous flow*. For the liquids considered in the previous section the two methods of estimating ΔE_{vis} give the same results, and as $V(\partial E/\partial V)_T$ and ΔE_{vap} have essentially the same magnitude and temperature dependence at atmospheric pressure. However, at the high pressures considered in the next section, the two quantities are quite different. Figure 11 illustrates the fact that

the pressure at which $-\Delta E_{\text{vap}}$ is a *maximum* is the point at which $(\partial E/\partial V)_T$ equals zero. Hence, if ΔV is the extra volume required for the flow process, the heat of activation in the two cases will be

$$\Delta H_{\text{vis}} = \Delta E_{\text{vap}}/n + P\Delta V \quad (100)$$

and

$$\Delta H_{\text{vis}} = (\partial E/\partial V)_T \Delta V + P\Delta V \quad (101)$$

depending on which process most nearly approximates the process of forming the cavity for flow. Both equation 100 and equation 101 have been applied to data at high pressures.

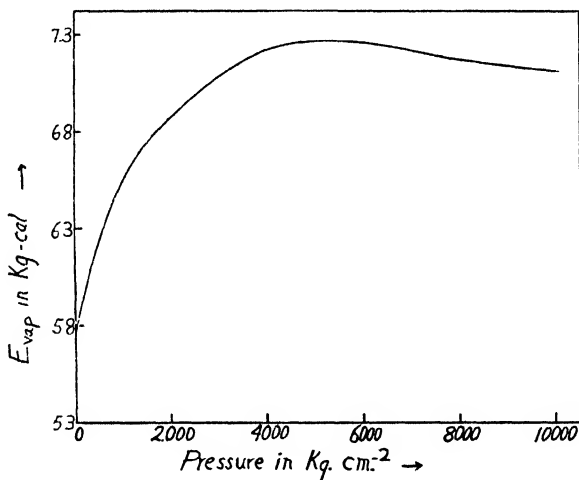


FIG. 11. The energy of vaporization of *n*-pentane at 30°C. as a function of the external pressure.

B. The effect of pressure on viscosity

The application of external pressure has the greatest effect on the rate of those reactions in condensed phases which have the greatest increase in volume resulting from the formation of the activated complex from the reactants. Since the free-energy difference between the normal and activated states for viscous flow in normal liquids results almost entirely from this increase in volume, it is not surprising that viscosity has a greater variation with pressure than any other property of pure liquids which has been studied.

In order to test equation 101, ΔV is identified with V/n , where n is the value required to give the correct temperature coefficient of viscosity.

This is equivalent to assuming that there is a linear relation between the size of the hole and the energy required to form it, and thus an n equal to 3 means that a cavity just one-third the size of the molecule is required.

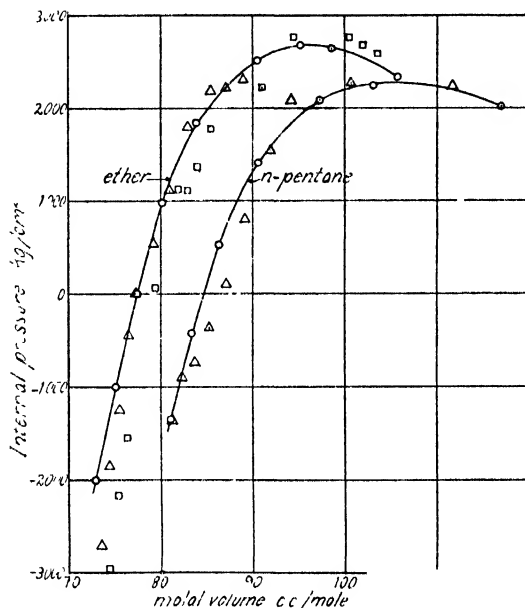


FIG. 12. Plots of internal pressure *versus* molal volume for ether and *n*-pentane (16). The circles are values computed from equation 102, using Bridgman's data for viscosity under pressure (6). The triangles and squares are values computed from the thermodynamic equation $P_i = (\partial E/\partial V)_T = T(\partial P/\partial T)_V - P$, using Bridgman's newer (1931) and older (1914) compression data, respectively (6).

If, following Hildebrand (28), we designate $(\partial E/\partial V)_T$ as P_i (the internal pressure), we have from equations 79, 84, and 101

$$\eta = 7.71 \times 10^{-4} [M^{1/2} T^{2/3} / V^{5/3} (P_i + P)]$$

$$\exp[(P_i + P)V/nRT] \exp(-\Delta S'/R) \quad (102)$$

Equation 102 may be most readily tested by making P_i the unknown and using observed values of viscosity to compute internal pressures for comparison with those obtained directly from P - V - T data. Figure 12 illustrates the application of this method, using Bridgman's data (6).

Equation 102 fails when applied to certain other liquids, notably mercury, and a viscosity-pressure equation based on equation 100 has proven to be more generally useful. This equation, giving the heat of activation

for viscous flow in terms of the energy of vaporization and the work against the external pressure, has been tested by Frisch, Eyring, and Kincaid (25). The expression employed for ΔH_{vis} is

$$\Delta H_{vis} = \Delta E_{vap}/n + PV/n' \quad (103)$$

Here ΔE_{vap} is the energy of vaporization at the particular pressure considered, and V/n' is the extra volume required for viscous flow. It is found that n' does not have the same value as n , being somewhat greater.

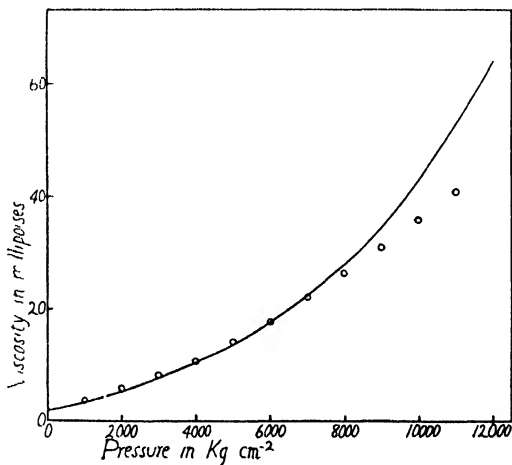


FIG. 13. Observed viscosities of ether (solid curve) as a function of pressure compared with calculated values (circles) computed on the internal pressure hypothesis (equation 102).

If the sound velocity method of getting the free volume is used, the equation giving the viscosity incorporating equation 103 may be written

$$\eta = (\pi RMT)^{1/2} (u_g/u_l) N^{-1/3} V^{-2/3} \exp(\Delta E_{vap}/nRT + PV/n'RT) \quad (104)$$

Here u_g is the velocity of sound in the gas, u_l is the same quantity for the liquid, and the other quantities have been defined. The procedure employed in testing equation 104 was to use the experimental viscosity, the energy of vaporization, the sound velocity in both the liquid and the gas, and the other terms on the right-hand side of equation 104 to determine the n giving the proper temperature variation of viscosity at atmospheric pressure. The values of n obtained in this way, given in table 8, are then employed in equation 104 in order to obtain values of n' at a given temperature and different pressures. A summary of the results for n' is given in table 9.

The values of n given in column three of table 7 are slightly different from those of Ewell and Eyring, because a different evaluation of the free volume has been used. Column six of table 8 shows that the value of n which gives the right temperature coefficient of viscosity also gives the absolute value to within an average value of 2 for liquids other than those classed as "hydrogen-bonded."

The individual computations of n' are shown in table 9. The treatments possible for benzene and for isopentane warrant giving only one value for

TABLE 8

Values of n which give the proper temperature variation of viscosity at atmospheric pressure (25)

SUBSTANCE	n (Ewell and Eyring (16))	n (Frisch, Eyring, and Kincaid (25))	n' MEAN VALUE	n'/n (Frisch, Eyring, and Kincaid (25))	η (OBSERVED) η (COMPUTED)
<i>n</i> -Pentane	4	4.4	7.8	1.8	1.1
Ethyl ether.....	4	4.5	7.8	1.6	1.9
Benzene	3	3.3	5.5	1.7	0.6
Isopentane		4.4	8	1.8	1.9
Mercury.....	20	11	23	2.1	2.8

TABLE 9

Values of n' as computed from the data at various pressures

PRESSURE	<i>n</i> -PENTANE AT 30°C.	DIETHYL ETHER AT 52.5°C.	BENZENE AT 25°C.	ISOPENTANE AT 50°C	MERCURY AT 0°C.	WATER AT 0°C.
<i>kg. per cm.²</i>						
1,000	6.0		5.5	8		124
2,000	6.9	4.7			25.0	14
4,000	8.5	6.0			23.0	8.0
6,000	9.7	6.8			21.2	(5000)7.2
8,000	10.4	7.2				
10,000	10.8	7.6				
12,000		7.9				

n' . It will be noted that n' for *n*-pentane, ether, and mercury does not vary greatly over the entire experimental pressure range, but that n' for water ranges from 124 at 1000 *kg. per cm.²* to 7.2 at 5000 *kg. per cm.²* This variation is interpreted as follows: At low pressures water has an open, 4-coördinated structure (3), and no extra volume is required for the activated complex for flow to form. As the pressure is increased, the open structure collapses, and at high pressures the activated complex needs as much extra space to form as is required by any other non-spherical

molecule. It is probably incorrect to assume that n is constant for these liquids at one temperature and varying pressures, but the value of n' is not greatly affected by a considerable change in n . Table 10 illustrates that the n' values computed for water using different n 's converge to about the same limit at high pressures.

The fifth column of table 8 gives the ratio of the mean value of n' over the pressure range to the value of n . The fact that this ratio is nearly constant and equal to 1.6 to 1.8 for the four non-metallic liquids in table 8 is of some significance. It has been previously shown that the energy required to form a hole in a liquid the size of a molecule is equal to the energy of vaporization. Although it might be reasonable to assume a linear relationship between the size of the hole formed and the energy required to form it, the fact that the ratio n'/n is not unity but 1.75 indicates that this may not be the case. Taking the data for ether as an example,

TABLE 10
Values of n' for water for different values of n at 0°C . (25)

P	τ	VALUES OF n'	
		$n = 4$	$n = 5.4$
kg. per cm. ²			
1000		16.1	124
2000	32	16	14
3000	19	11	9.7
4000	14	8.8	8.0
5000	10.6	7.7	7.2

we find an n' of 7, indicating that a hole approximately one-seventh the size of the molecule is required for viscous flow. However, the activation energy is two-ninths of the energy of vaporization. We accordingly have the interesting physical result that there is an energy of dissociation of large holes into smaller ones, i.e., two holes, each one-seventh the size of a molecule, cost considerably more energy than one hole two-sevenths molecular size. In the case of ether, seven holes, each one-seventh the size of a molecule, would liberate energy equal to fourteen-ninths of the energy of vaporization, on being combined into a single cavity of molecular dimensions.

The ratio n'/n for mercury does not differ greatly from that for the non-metallic liquids in table 8, but n and n' are themselves much greater. That the ratio of n' to n is again approximately 2 for a hole as small as one twenty-third the size of the atom is an interesting fact.

Figure 14 shows plots of observed and computed viscosities as a function

of pressure for ether, mercury, and *n*-pentane, with constant values of n and n' in equation 104. The agreement, while perhaps not as satisfactory as might be hoped for, is probably as good as can be expected.

It is clear that additional evidence is desirable before choosing between the two methods outlined above for treating the pressure variation of viscosity. The essential requirement is a simple, accurate method for securing the free energy of formation of a cavity in the liquid at any given temperature and pressure, and an entirely adequate treatment of viscosity

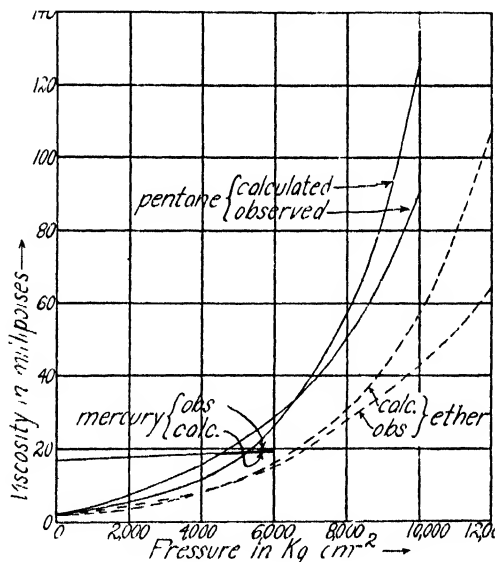


FIG. 14. Comparison of observed viscosities and those computed from equation 104. The values of n are 4.5, 12, and 4.4, and those of n' are 8, 23, and 10 for ether, mercury, and pentane, respectively.

under pressure cannot be given until this is available. The recent work of Kirkwood (39) may prove of value in this connection.

VII. APPLICATION TO VARIOUS CLASSES OF LIQUIDS

A. A classification of types of liquids

From the point of view of viscous behavior and of many other properties as well, Ewell (14) has given a classification of liquids which is essentially the same as that given below.

I. Those in which the forces between molecules are almost exclusively undirected:—(a) Relatively small molecules: e.g., carbon tetrachloride,

chlorine, argon, benzene. (b) Very long chain molecules: e.g., linear polymeric resins, such as polystyrene, polyisobutylene; very long chain hydrocarbons, such as some lubricating oils; selenium and μ -sulfur; raw rubber.

II. Those in which the cohesive forces are directed in part:—(a) Molecules containing dipoles: e.g., those with a single strong dipole making possible a weak association into pairs, e.g., ethyl chloride, ethyl bromide, acetone; those with two or more strong dipoles making possible a two- or three-dimensional network of dipole bonds, e.g., 1,5-dichloropentane, *p*-dinitrobenzene. (b) Molecules capable of forming hydrogen bonds,—e.g., water, phenol; in general, any liquid whose molecules have OH or NH groups. (c) Molecules capable of forming intermolecular covalent bonds, e.g., silicon dioxide, germanium dioxide, boron trioxide, beryllium fluoride, and all silicate, borate, and phosphate liquids which are not too basic.

III. Metallic liquids: molten metals.⁹

IV. Ionic liquids: molten salts.⁹

The applications of the theory thus far have been confined to examples chosen from types I(a), I(b), and II(a),—the so-called normal liquids. Although these are the ones to which the theory may be most easily applied, liquids of more complex structure may also be treated qualitatively, and a discussion of the applications to μ -sulfur and water will comprise the next two sections.

B. The viscosity of sulfur

As is well known, liquid sulfur is a fluid yellow liquid between the melting point and about 160°C. and also in the supercooled liquid region below the melting point. Above 160°C. the viscosity increases rapidly, increasing several thousandfold between 160° and 190°C., and thereafter the viscosity decreases in a normal way. Figure 15 shows the data of Rotinjan⁵ plotted as $\ln \eta$ against $1/T$. Rotinjan's data show that between 160° and 250°C. the viscosity of sulfur is a function of the time as well as the temperature, and the values given in that range are only rough averages and probably not equilibrium values.

It is seen that the plot has two linear portions, below 160° and 250°C., for which B is 7.04 and 18.35 kg.-cal. per mole, respectively. In the region below 160°C., x-ray evidence (66) indicates that the molecule is a puckered S_8 ring, and by comparing this structure with cyclohexane, it seems likely that n would be 3 for this type of molecule. The heats of vaporization of sulfur have been accurately measured by West and Menzies (67). Their value of ΔE_{vap} at 120°C. (the middle of this linear part of the plot) is 2.59 kg.-cal. per gram-atom or 20.7 kg.-cal. per mole of S_8 . This is very nearly

⁹ Groups III and IV are really subdivisions of Group I, but are classified separately for obvious reasons.

three times 7.04, the value of B in this range, and this may be considered as further evidence toward confirming the S_8 molecule in the liquid in this range.

In the range above 250°C . the much larger slope of the curve indicates that the molecule is much larger than it is below 160°C . This larger sulfur molecule might be either a large ring, a branched chain, or a long straight chain, of which the latter seems most likely to be the correct structure. By analogy with the straight-chain hydrocarbons, n should be 4 for this type of molecule, and this would indicate a value of ΔE_{vap} of $4 \times 18.35 = 73.4$ kg.-cal. per mole on our hypothesis. West and Menzies give 2.05 kg.-cal. per gram-atom for ΔE_{vap} at 350°C . (the middle of this linear part

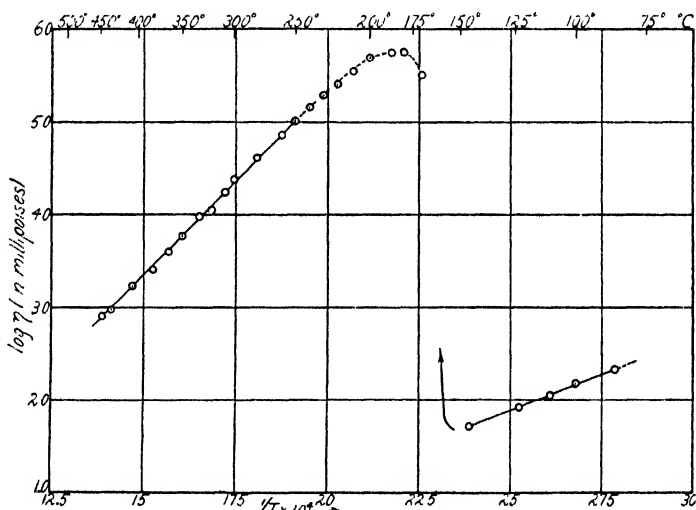


FIG. 15. Plot of $\log \eta$ versus $1/T$ for liquid sulfur from the data of Rotinjanz (16, 54)

of the curve) and, comparing this with the value deduced above, the molecular weight of the unit of flow is calculated to be S_{36} . In contrast to such a molecular weight of the unit of flow, the unit of vaporization is still S_8 , as shown by vapor density data (16).

This figure of 36 can be interpreted as an average chain length averaged over all the molecules in the liquid and averaged over the whole temperature range from 250° to 450°C . The alternative is that, although the chains may be longer, they flow in segments, the approximate number of atoms in the units of flow being 36. These segments, although tied together, jump as units. At any temperature there is probably an equilibrium mixture of chains of varying length, and, as the temperature is

raised, the average chain length will become smaller as the equilibrium constant changes. The linear relation between $\ln \eta$ and $1/T$ is not a sufficient condition for an unchanging molecular state in a liquid. Normal liquids composed of a single molecular species almost without exception give linear $\ln \eta$ versus $1/T$ plots. However, a liquid composed of an equilibrium mixture of several molecular species might also give a linear plot, since the equilibrium constant changes according to van't Hoff's equation, which is of the same form of temperature dependence as is the viscosity of a liquid.

The hypothesis had been advanced by Warren and Burwell (66) that the increase in viscosity above 160°C. is "probably due to the S_8 ring breaking open and forming irregular chains which tangle with one another and give rise to the marked increase in viscosity". These results give a more concrete form to this idea of long chains and obviate the necessity of postulating the indefinite concept of tangling of chains, since any liquid composed of chains averaging 36 atoms in length or more, e.g., a hydrocarbon, will be a very viscous liquid. The fact that liquid sulfur is not a normal close-packed liquid is shown by the work of Gingrich (26), who found that the liquid had about two nearest neighbors at the normal covalent bond distance, whereas liquid sodium or mercury has about eight nearest neighbors. This fact indicates that the molecule in the liquid sulfur is either a chain or a ring.

C. The viscosity of water and other associated liquids

Liquids belonging to Group II(b) of the above classification are ordinarily called abnormal or associated liquids. Among other anomalies these liquids, composed of molecules containing OH or NH groups, have much higher viscosities than would be expected from the size and structure of the molecules. For instance, water is much more viscous than hydrogen sulfide or methane; ethyl alcohol and ethylamine are much more viscous than propane; aniline and phenol are more viscous than toluene; etc. This abnormally large viscosity is due to the hydrogen-bond structure of these liquids. According to present concepts,³ for instance, each oxygen atom in the water molecule in ice is surrounded by four hydrogen atoms at approximately tetrahedral angles. Two of these four hydrogen atoms are bound to the central oxygen atom by primary valence forces and are a distance of 1.0 Å. away. The other two are "hydrogen bonded" at a distance of 1.8 Å. Ice at low temperatures has its maximum coordination of 4, i.e., there are four hydrogen bonds binding the water molecule to its neighbors, two through the oxygen and one for each hydrogen atom in the water molecule. Water at the melting point at 1 atmosphere pressure is still coordinated to some extent, but the degree of coordination is probably

somewhat below the maximum value of 4. Further, the degree of coördination¹⁰ will probably change with the temperature and pressure. Other liquids containing OH and NH groups are likewise thought to possess hydrogen-bonded structures to some extent. When viscous flow takes place in these liquids, not only must van der Waals' cohesion be overcome, but hydrogen bonds must be broken as well. Table 11 shows that $\ln \eta$ versus $1/T$ is not a straight line for water but that B decreases rapidly as the temperature is raised and, further, that the ratio $\Delta E_{\text{vap}}/B$ increases with the temperature. Ewell and Eyring (16) have interpreted the rapid decrease of η and B with rising temperature as due to a decrease in the number of hydrogen bonds that have to be broken for the flow process to take place. This conclusion is consistent with the observation (41, 65) that D_2O is about 25 per cent more viscous than H_2O . Since it requires more energy to break the deuterium bond than the hydrogen bond, and

TABLE 11
The energy of activation for viscous flow of water

t	η	B	ΔE_{vap}	$\Delta E_{\text{vap}}/B$
°C.	mills poises	kg.-cal. per mole	kg.-cal. per mole	
0	17.95	5.06	10.18	2.0
50	5.49	3.42	9.62	2.8
100	2.84	2.80	8.98	3.2
150	1.84	2.11	8.28	3.9

since this term occurs as an exponential in the viscosity formula, it is not surprising to find so great a difference.

Similar considerations apply to other associated liquids. Since the maximum possible average coördination is equal to twice the number of OH or NH groups in the molecule, monohydric alcohols have a maximum coördination of 2, and the fact that ethyl alcohol and water have about the same viscosity indicates that ethyl alcohol probably possesses a large fraction of its maximum 2-coördination.

Ethylene glycol has a maximum average coördination of 4, the same as water, and the fact that glycol is about twenty times as viscous as water or as alcohol indicates that the degree of coördination is much higher in glycol than in water or alcohol. This is probably due to the fact that the two OH groups are separated in glycol, giving less interference between the hydrogen bonds attached to the two groups. Similarly, glycerol has a maximum coördination of 6, so that as little as half of the maximum

¹⁰ The degree of coördination is used here only in the sense of the average number of hydrogen bonds per molecule.

coördination will permit a three-dimensional network of hydrogen bonds, giving rise to the high viscosity of glycerol. Quantitative work along the lines suggested qualitatively in this section should throw light on the question of the contributions to the cohesive energy of liquids made by van der Waals, dipole, and hydrogen-bond forces.

D. The viscosity of liquid metals

Most metals give linear $\ln \eta$ versus $1/T$ plots, just as normal covalent liquids do. The most striking fact regarding the metals is the large value of the $\Delta E_{\text{vap}}/B$ ratio, which ranges from 8 to 25, as compared to 3 or 4 for normal liquids. This low activation energy for flow is consistent with the conclusion that the unit involved in flow is much smaller than the

TABLE 12
The energy of activation for viscous flow in liquid metals

METAL	MIDPOINT OF TEMPERATURE RANGE	ΔE_{vap}	B	$\frac{\Delta E_{\text{vap}}}{B}$	$\frac{\Delta E_{\text{vap}}}{B} \times \left(\frac{r_{\text{ion}}}{r_{\text{atom}}}\right)^3$
	°C.	kg.-cal. per mole	kg.-cal. per mole		
Na	500	23.4	1.45	16.1	2.5
K	480	19.0	1.13	16.7	3.4
Ag	1400	60.7	4.82	12.5	3.8
Zn	850	26.5	3.09	8.6	2.1
Cd	750	22.5	1.65	13.5	4.0
Hg	250	13.6	0.65	20.8	2.4
	600	12.3	0.55	22.2	2.5
Ga	800	34.1	1.13	30.3	2.5
Sn	600	15.3	1.44	10.6	4.1
	1000	14.5	1.70	8.6	3.3
Pb.	700	42.6	2.80	15.9	5.0

unit of vaporization. The unit of vaporization being the atom, the unit involved in flow is presumably the much smaller metal ion, i.e., the atom partially or completely stripped of its valence electron or electrons.

On this assumption an approximate value for the energy of activation is given by

$$B = \frac{\Delta E_{\text{vap}}}{n} \times \frac{\text{volume of ion}}{\text{volume of atom}} \quad (105)$$

where n has its usual value for normal liquids.

Table 12 shows the experimental values of the quantity

$$\frac{\Delta E_{\text{vap}}}{B} \times \frac{\text{volume of ion}}{\text{volume of atom}}$$

using the ionic atomic radii given by Wyckoff (70) to determine the volumes of the ion and atom, respectively. When the temperature variation of the energy of vaporization was known, a value was taken arbitrarily in the middle of the range over which the viscosities were measured. For the polyvalent metals the following were assumed to be the flowing ions: Hg^+ , Sn^{++} , Pb^{++} .

It is seen that the values of n given in column 6 cluster around the average value of about 3. While this result is interesting and suggestive, the situation is complicated by the nature of the bonding in metals. The theorem that the energy of forming a hole the size of a molecule equals the energy of vaporizing a molecule is only proved in the case where a bond between pairs is independent of the position of other atoms. It is by no means clear that such a theorem holds for a substance containing conducting electrons.

As we have already seen, ordinary liquids at high pressures obey the inequality $V(\partial E/\partial V)_T < \Delta E_{\text{vap}}$. This same inequality holds for metals even at atmospheric pressure, so that B should perhaps be compared to $V(\partial E/\partial V)_T$, rather than to ΔE_{vap} . Because of the above inequality, such a comparison would reduce the value of n required to reproduce the temperature coefficient of viscosity. The data are, in general, not available for such a comparison, but for mercury $V(\partial E/\partial V)_T/B$ equals 4.5. The qualitative concept of the metal ions moving short distances without their valence electrons is the counterpart of the theory of conducting electrons moving short distances without disturbing the ions.

E. Mixture law for viscosity

We give here the discussion of Powell, Roseveare, and Eyring (52). The viscosity of a mixture of liquids is not related to the viscosities of the pure components by any simple additive relation. Several equations have been tested in the search for an additive function for viscosity, among them being the following (*cf.* references 1, 4, 34, 35):

$$\varphi = N_1\varphi_1 + N_2\varphi_2 \quad (106)$$

$$\varphi^{1/2} = N_1\varphi_1^{1/2} + N_2\varphi_2^{1/2} \quad (107)$$

$$\varphi^n = N_1\varphi_1^n + N_2\varphi_2^n \quad (108)$$

$$\log \varphi = N_1 \log \varphi_1 + N_2 \log \varphi_2 \quad (109)$$

the weighting being done according to weight fraction, volume fraction, and mole fraction. There is also a wide variety of equations containing one or more adjustable constants. For example, an equation of the type of equation 108 has been used by petroleum engineers to estimate the viscosity of mixtures of lubricating oils: for high-viscosity paraffin base

plus low-viscosity naphthene base, the exponent is $1/30$; for the opposite case, the exponent is $1/3.1$; for two oils of the same base, the exponent is $1/6.5$ (69). Such equations are convenient for interpolation purposes, but that they fit the experimental data is more a matter of arithmetical inevitability than of merit. Of the equations not containing an adjustable constant, equation 109 fits the experimental data rather better than the others.

If the flow process were strictly determined by the properties of one molecule flowing, equation 106 would be expected to hold. If it were determined by the properties of two molecules flowing past each other, equation 107 would be expected to hold. However, it is probable that the cheapest way for a hole to be made is for the flowing molecule to squeeze against its neighbors, which in turn squeeze against their neighbors, until the over-all result is the expansion of the entire liquid. Thus the average thermodynamic properties of the entire liquid may be involved when any individual molecule flows.

As a simple approximation, equation 76 may be used for mixtures if for V is inserted the actual value of the average molal volume, and for ΔF^\ddagger is inserted the weighted arithmetical mean of the values for the pure components. Thus

$$\phi = \frac{V}{N\bar{h}} \exp \frac{-(N_1\Delta F_1^\ddagger + N_2\Delta F_2^\ddagger)}{RT} \quad (110)$$

When V_1 and V_2 are not too different, equation 110 reduces to equation 109.

As a test of equation 110, ΔF^\ddagger has been plotted against mole fraction for a number of pairs of liquids. Three types of curves are obtained: (a) Closely similar liquids, e.g., benzene and anisole, give a straight line. (b) Liquids which definitely form a compound, e.g., chloroform and ether, give a convex curve. (c) Liquids which are slightly dissimilar give a slightly concave curve; liquids which are markedly dissimilar, e.g., benzene and alcohol, give a markedly concave curve.

It has been pointed out (15) that the deviations from a linear fluidity law are roughly parabolic and are symmetric about the 50 mole per cent line. The same behavior is noted in the deviations of ΔF^\ddagger from a linear law. ΔH^\ddagger , on the contrary, always shows larger deviations, which are usually not symmetrical and may even change sign. These large deviations shown by ΔH^\ddagger tend to be counterbalanced by entropy changes.

It was early remarked that non-aqueous liquid pairs showing a minimum in the viscosity curve also showed a negative deviation from Raoult's law, and those showing a maximum in the viscosity curve showed a positive deviation from Raoult's law (72). In order to formulate this quantitatively, the deviations of ΔF^\ddagger from a linear law (calories at 50 mole per cent)

were plotted against the deviations from Raoult's law (calories at 50 mole per cent) for systems for which partial pressure data are available or can be estimated. The curve resembles closely that in figure 6, in that the points tend to fall along a line drawn through the origin with a slope of $1/2.45$. The mixture law of equation 110 is therefore to be modified to read

$$\varphi = \frac{\bar{V}}{N\bar{h}} \exp \left(- \left[(N_1 \Delta F_1^\dagger + N_2 \Delta F_2^\dagger) - \frac{\Delta F^E}{2.45} \right] / RT \right) \quad (111)$$

where ΔF^E is the excess free energy of mixing (55, 56).

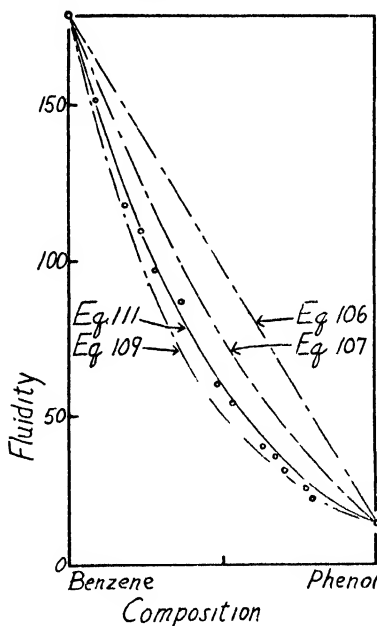


FIG. 16. Plots of various fluidity-composition equations for binary liquid mixtures applied to the system benzene-phenol, compared with observed fluidity values shown by circles.

The application of this mixture law is illustrated in figure 16, where the experimental values for the fluidity of the system benzene-phenol are plotted, together with curves calculated according to several different mixture laws.

F. Relation of fluidity to volume

Batschinski (2) has shown that, for a large number of liquids, there is a linear relationship between volume and fluidity. The data of Bridgman at high pressures (6), which show that, for non-associated liquids, the

temperature coefficient of fluidity at constant volume is insignificant compared to the temperature coefficient at constant pressure, indicate also that the fluidity of normal liquids under ordinary conditions is nearly a function of volume alone. Such linearity indicates that the theory of fluidity is fundamentally linked to the theory of liquid volume, and has led Powell, Roseveare, and Eyring (52) to the idea of a number of holes in the liquid which shows a variation approximately proportional to the volume change.

Cernuchi and Eyring (7) have considered liquids as being made up of a binary mixture of molecules and holes having the same volume as molecules. Kirkwood (39) pointed out that the theory of holes leads to results incompatible with critical data when the holes are the same size as the molecules. The temperature and pressure coefficients of viscosity indicate that the volume of a hole necessary for flow is a small fraction of the size of a molecule (25).

Powell, Roseveare, and Eyring (52) assumed that a liquid is a solution of holes and molecules and that the size of the holes is a characteristic of the material. The fusion of n molecules of a substance is the dissolving of n_h holes such that the entropy of solution is two units, or

$$\Delta S = 2 = -nk \ln \left(\frac{n}{n + n_h} \right) - n_h k \ln \left(\frac{n_h}{n + n_h} \right) \quad (112)$$

since the observed entropy of fusion is about two entropy units for a large number of monatomic substances (30). For one mole of substance $n = N$ and then, from equation 112, $n_h = 0.54N$. The solution of 0.54 mole of holes in 1 mole of a substance gives the required disorder entropy to change a solid into a liquid.

If the volume of a hole be taken as $1/h$ of the volume of a molecule, and v_s and v_l are the respective molecular volumes of the solid and liquid at the freezing point, then we have

$$r \frac{v_l - v_s}{v_s} = 0.54 \quad (113)$$

For a number of substances $\Delta V/V$ for fusion is approximately 0.1, making r about 5 or 6. Most metals have a value of r between 20 and 25. The result that a new equilibrium position has a volume about one-sixth that of the non-metal molecule and about one twenty-third the metal molecules is exactly the result found by Frisch, Kincaid, and Eyring (25) from the effect of pressure on viscosity. Thus this model relates quantitatively two otherwise apparently phenomena, i.e., melting and viscous flow. The experimental value of the volume of the solid in equation 113 has significance only if the short-range order of structure of the liquid is the same

as that of the solid. Water has a negative value of $\Delta V/V$ on melting, owing to a change in coordination number on melting.

For substances to which equation 113 may be applied, Powell, Roseveare, and Eyring find that, at the melting point, their fluidity behavior is better described by the partition function for the solid state for the extra degree of freedom possessed by the normal molecule, than by the liquid partition function, obtained from the gas function modified by the introduction of free volume.¹¹

For temperature ranges extending not too far above the normal boiling points, they would write, in place of equation 96,

$$\varphi = \frac{V_1}{N h} \frac{\Theta Z r v_1 - v_1}{T v_1} \exp(-\Delta E'/RT) \quad (114)$$

In equation 114, Z is a numerical constant related to the coordination number; $\Delta E'$ is the energy of activation at constant volume¹² (in place of that at constant pressure in equation 96); Θ , the Debye characteristic temperature, while not often known, can be estimated by the relation (40)

$$\Theta = A \left[\frac{T_m}{M V^{2/3}} \right]^{1/2} \quad (115)$$

where A is a numerical constant, T_m is the melting temperature, and M is the molecular weight.

For many liquids the energy of activation for constant volume is very small, and when this is the case, there will be a temperature range in which the variation of the exponential term will compensate the $1/T$ factor, and there will result the frequently observed linear relation between φ and V_1 .

When $\Delta E'$ is large, as in the case of the higher alcohols, this is no longer the case. In figure 17 is shown a plot of fluidity against volume for isopropyl alcohol for which $\Delta E'$ is large. Data of Bridgman at intermediate pressures permit the estimation of the heat of activation at constant volume, yielding a value of 3900 calories. The result of plotting $\varphi T \exp(3900/T)$ against volume should be a straight line, which is shown to be the case in figure 17.

As another test of equation 114, $\log \varphi$ is plotted against $1/T$ in figure 18 for benzene. A number of such graphs may be found in the paper of Sheppard and Houck (58). The resulting curved line for benzene in

¹¹ Actually a combination of solid and gas partition functions is being investigated. This should approximate the function for the solid at the melting point for normal substances, and should approach the function for the gas at the critical temperature.

¹² The energy of activation at constant pressure may be thought of as principally that energy required to form the hole necessary for flow, while that at constant volume is merely the (usually) small energy required to activate the molecule for flow into a hole already present.

figure 18 is to be compared with the straight line which results from plotting $\log [\phi T / (V_i - V_s)]$ against $1/T$. In the case of many substances the data available are in a temperature range where both plots are straight lines.

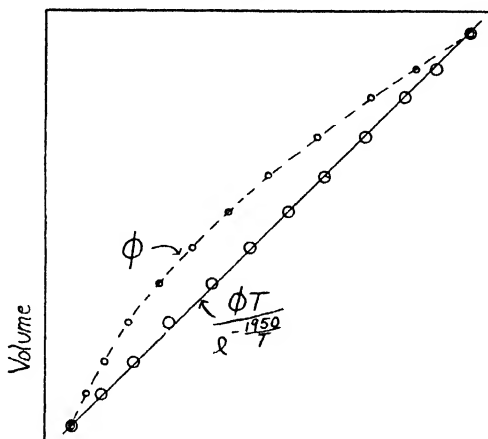


FIG. 17. Plots of volume *versus* fluidity (small circles) and $\phi T \exp (1950/T)$ (large circles) for isopropyl alcohol.

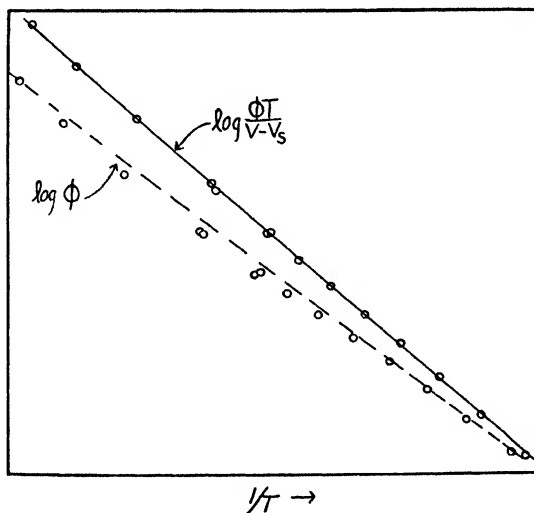


FIG. 18. Comparison of linearity of plots of $1/T$ against $\log \phi$ (broken line) and against $\log \phi T / (V - V_s)$ (solid line) for benzene. The temperature range involved is from about 280° to 460°A .

The linear relation between ϕ and V_i found for substances with small values of $\Delta E'$ holds not only for volume change due to temperature varia-

tion but also for that due to pressure variation through fairly wide pressure ranges. So long as the pressure has very little effect on the liquid structure other than to squeeze out holes, the linear relation holds. For very high pressures, fluidity decreases less rapidly with increased pressure than the linear law predicts. Figure 19 shows plots of fluidity against volume at constant temperature for pressures up to 12,000 kg. per cm.² for ether and for ethyl alcohol.

G. The flow of large molecules

For the viscous flow of hydrocarbons, Ewell and Eyring (16) have found that the heat of activation is usually very close to one-fourth the heat of

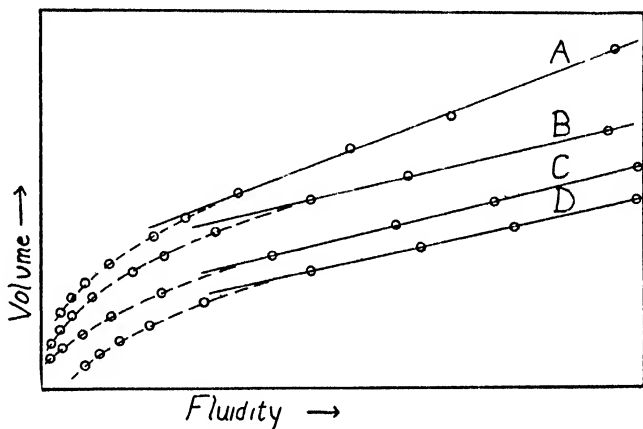


FIG. 19. Showing the relation of fluidity to volume at high pressures. Curve A is for ether at 75°C., curve B for ether at 30°C. Curve C is for ethyl alcohol at 75°C., and curve D for ethyl alcohol at 30°C. Abscissae are arbitrary. The break from linearity seems to occur at pressures between 2000 and 3000 atmospheres. The fourth point from the right in each curve is for 2000 atmospheres.

vaporization of a molecule the size of the unit of flow. Kauzmann and Eyring (33), on plotting ΔH^\ddagger of viscous flow for normal hydrocarbons against chain length, found that, for chains above about twelve atoms in length, there is a significant and increasing deviation from the relationship

$$\Delta H^\ddagger = (1/4)\Delta H_{vap}$$

for the molecule. This is taken to mean that chains longer than about twelve atoms do not move as a unit, but move in segments. Furthermore, there is an indication that, as the chain length increases beyond about fifty atoms, ΔH^\ddagger is independent of the total chain length, showing that, for molecules of this length, the size of the segments which move is independent of the length of the molecule. From the limiting values of ΔH^\ddagger thus

found, it is estimated that the segments involved in the flow of long hydrocarbon molecules are, on the average, twenty atoms in length.

An analogous treatment of results recently reported by Flory (23) for the behavior of polymers reveals that segments averaging about thirty atoms in length are involved. In rubber the segments are about forty atoms long, while in plastic sulfur they are about twenty atoms long.

Although the temperature variation of the viscosities of long-chain polymers is determined solely by the nature and size of the segments of which they are composed, there is definite evidence that a further factor, depending on chain length but not on temperature, operates to make long chains more viscous than short chains. Thus, in connection with the viscosities of the normal paraffins, long chains are definitely less fluid than would be expected from the recent extension of the hole theory of liquids. Flory's work indicates that the viscosities of long-chain molecules are proportional to $\exp(a\sqrt{Z})$, where Z is the chain length of the entire molecule and a is a temperature-independent constant.

This behavior is readily understood when it is realized that, although the segments in a large molecule are moving about just as rapidly as those in a small molecule, the movements of the segments of the large molecule must be coördinated to a far greater extent than those of a smaller molecule, in order for the molecule to move forward a given distance.

According to Kauzmann and Eyring (33), the exponential form found by Flory follows if we say that, on each jump by a segment, there is a chance of failure of $aZ^{1/2}/n$, where n is the number of segments in the molecule. The chance of success in a single jump by one segment is then $1 - \frac{aZ^{1/2}}{n}$, and the chance of successful jumps by n segments is

$$\left[1 - \frac{aZ^{1/2}}{n}\right]^n$$

But the fluidity is proportional to the fraction of successful jumps, so that

$$\varphi = K \left[1 - \frac{aZ^{1/2}}{n}\right]^n \quad (116)$$

Since

$$\left(1 - \frac{x}{n}\right)^n = \exp(-x)$$

when $n \gg x$, we have

$$\eta = 1/\varphi = K' \exp(a\sqrt{Z}) \quad (117)$$

VIII. DIFFUSION PROCESSES IN LIQUIDS

Although the viscosity of liquids has been extensively studied, data for diffusion in liquids are very meager. Thus the measurements of Orr and Butler (48) are the only ones available for testing the applicability of equation 66 for self-diffusion. Taking their values for the diffusion of heavy into light water at 0°C. and 45°C. and utilizing the data for the viscosity of ordinary water at the same temperatures, Eyring (19) has employed equation 66 to obtain values of the ratio $\lambda_1/\lambda_2\lambda_3$ of the dimensions of the diffusing molecule. This result may be combined with the product $\lambda_1\lambda_2\lambda_3 (= V/N)$ to yield a value of λ_1 equal to 1.44 Å. at 0°C. and 1.47 Å. at 45°C. At these same temperatures $(\lambda_2\lambda_3)^{1/2}$ equals 4.54 and 4.50 Å., respectively. This result is seen to be in accord with the principle that reactions will proceed by all possible mechanisms and therefore chiefly by the fastest ones when it is recalled that λ_1 is the dimension of the flowing molecule perpendicular to the plane of shear. It appears probable that in diffusion, as in viscous flow, the fastest process will be one in which the plane of the water molecule tends to coincide with the plane of flow, i.e., that λ_1 will be the thin dimension of the molecule. In the calculation, the viscosity of water has been used, whereas a value intermediate between that of H₂O and that of pure D₂O should have been used. This, however, would be a small correction, having no effect on the general conclusion. That the Stokes-Einstein diffusion equation is not applicable to this case is seen by the unreasonably small value of 1.46 Å. ($(V/N)^{1/3} = 3.1$ Å.) that it yields for the diameter of the diffusing molecule when the data at 45°C. are applied in equation 39. The dimensions of the water molecule, as determined from Fischer-Hirschfelder models, are $2.3 \times 4.0 \times 3.0$ Å., thus being in rough agreement with the dimensions derived from diffusion. There is no reason for expecting the Stokes-Einstein equation to be applicable here, since it is derived with the condition that the diffusing particle be so large in comparison to the solvent molecules that the solvent may be considered to be continuous.

Equation 65 may be rewritten in the form (61)

$$D = \lambda^2 \frac{kT}{h} \exp \left[\frac{-\Delta F^\ddagger}{RT} \right] \quad (118)$$

If we assume that the degree of freedom corresponding to flow is a translational one, and that the partition functions for other degrees of freedom are the same for the initial and activated states, then equation 118 may be transformed to

$$D = \lambda^2 \frac{kT}{h} \frac{h}{(2\pi mkT)^{1/2} v_f^{1/3}} \exp(\Delta H^\ddagger/RT) \quad (119)$$

$$= \frac{\lambda^2}{v_f^{1/3}} \left(\frac{kT}{2\pi m} \right)^{1/2} \exp(-\Delta E_{\text{vap}}/nRT) \quad (120)$$

where v_f is the free volume. For diffusion processes, just as for viscous flow, a hole must be provided to diffuse into. The energy necessary will involve some fraction of the energy of vaporization so that, in equation 120, we write $\Delta E_{\text{vap}}/n$ for ΔH^\ddagger . Although diffusion is a rate process and should thus show an exponential variation with temperature, the data have normally been represented as a linear function of temperature. However, the precision measurements of Cohen and Bruins (8) on the diffusion of tetrabromoethane in tetrachloroethane extending over the temperature range 0° to 51°C . could not be fitted with a linear interpolation formula, and these authors employed a quadratic formula to represent their measurements. Taylor (63), using the results of Cohen and Bruins, plotted $\log D$ against $1/T$ and obtained an excellent linear plot, from which an activation energy of diffusion equal to 3500 calories was obtained. This value lies

TABLE 13
Diffusion of tetrabromoethane in tetrachloroethane

TEMPERATURE	$\lambda \times 10^4$	$v_f^{1/3} \times 10^3$	ΔE_{vap}	$D \times 10^6$ (OBSERVED)	$D \times 10^6$ (CALCULATED)	$D_{\text{obad}}/D_{\text{calcd.}}$
$^\circ\text{A.}$			kg.-cal. per mole			
273.4	3 124	6 45	9 852	0.351	0.64	0.55
280 7	3 140	6 69	9 784	0.419	0 77	0 54
288	3.155	6.92	9.719	0 497	0 92	0 54
298	3.174	7.25	9 624	0.611	1.14	0.54
308 6	3 195	7 59	9 525	0 741	1.42	0.52
324.1	3.230	8.13	9 383	0.954	1 89	0.51

between that for viscous flow in tetrachloroethane, 3000 calories, and that for viscous flow in tetrabromoethane, 3750 calories.¹³

Stearn and Eyring (61) have used the results of Cohen and Bruins as a test of equation 120, employing average values for the quantities occurring in equation 120, based on a liquid mixture containing 7.83 mole per cent of tetrabromoethane studied by Cohen and Bruins. For λ and $v_f^{1/3}$ they took the weighted arithmetical mean, and for ΔE_{vap} the weighted geometric mean. Their results are given in table 13. The constancy of the ratio $D_{\text{obad.}}/D_{\text{calcd.}}$ shows that the calculated values of D reproduce the experimental temperature coefficient very closely. In agreement with Taylor (63) they took $n = 3$. (The value $n = 2.65$ would have reproduced the experimental results almost exactly.)

The data of Scheffer and Scheffer (57) on the diffusion of mannitol in

¹³ Value estimated from the boiling point, using the same Trouton's constant and value of $\Delta E_{\text{vap}}/B$ as for tetrachloroethane.

aqueous solutions in the temperature range 0° to 70°C . give activation energies varying slightly with temperature. The variations are of the same nature already noted for the viscous flow of water, probably owing to the hydrogen-bonded structure of the solution. However, when $\log D$ is plotted against $1/T$, the only point far off from the straight line giving an average slope is that for 0°C . The average slope gave $\Delta E_{\text{act}} = 4047$ calories, and this, with the average value of ΔE_{vap} for water between 0° and 70°C ., gives a value of $n = 2.4$. In table 14 are given the results of Stearn and Eyring, using equation 120 to calculate D . They took ΔE_{vap} for water as 9700 calories and n equal to 2.4. The constancy of the ratio $D_{\text{obsd}}/D_{\text{calcd.}}$ is again noted.

Although data for the temperature variation of diffusion are scanty except for measurements at two temperatures rather close together, nevertheless, on the basis of these data, Öholm (47) pointed out that substances showing a high value for the diffusion coefficient always showed a small

TABLE 14
Diffusion of mannitol in aqueous solutions

TEMPERATURE	$D_{\text{obsd.}} \times 10^6$	$D_{\text{calcd}} \times 10^6$	$D_{\text{obsd.}}/D_{\text{calcd.}}$
273.0	0.26	0.26	1.0
296.2	0.61	0.43	1.4
305.9	0.75	0.52	1.4
316.4	0.97	0.63	1.5
325.3	1.14	0.74	1.5
335.0	1.35	0.89	1.5
343.2	1.56	1.05	1.5

temperature coefficient. This generalization is exemplified in the data in table 16, where values of the temperature coefficient α , defined by $D_2/D_1 = 1 + \alpha(T_2 - T_1)$, are to be compared with those of D . Such a relation is to be expected on a reaction rate theory of diffusion. If a series of reactions do not have greatly varying entropies of activation, then the slowest ones, as well as those with the largest temperature coefficients, will be those having the largest energy of activation. The generalization of Öholm on the diffusion process is analogous to the observation of Kohlrausch on ionic mobilities. These ions with the greatest ionic mobility have the smallest temperature coefficients of mobility. This is also the reason that the transport numbers of ions tend to approach 0.5 as the temperature is raised. The more slowly moving ions with transport numbers less than 0.5 at a given temperature will have greater temperature coefficients of mobility than the rapidly moving ones with transport numbers greater than 0.5.

A further test of equation 120 is given in tables 15 and 16, taken largely from Stearn, Irish, and Eyring (61). The results are grouped into three classes: aqueous diffusion, diffusion of different solutes in the same non-aqueous solvent, and diffusion of the same solute in different non-aqueous solvents. In table 16, values of $D\eta$ are given in order to test the validity of the Stokes-Einstein relation (equation 69), according to which $D\eta$ should be constant at constant temperature. The variation in $D\eta$ is much less than in D , but, applying equation 69 to values of $D\eta$, variation from two- to

TABLE 15
Diffusion data for different solutes in the same solvent

SOLUTE	$D_{\text{obsd}} \times 10^6$	$D_{\text{calcd}} \times 10^6$	$D_{\text{obsd.}}/D_{\text{calcd}}$	$D \left(\frac{V_{\text{solution}}}{N} \right)^{1/3}$
Diffusion in aqueous solutions at 18-20°C.				
Methyl alcohol	1.37	0.39	3.5	555
Ethyl alcohol	1.10	0.38	2.9	504
<i>n</i> -Propyl alcohol	0.98	0.35	2.8	488
<i>n</i> -Butyl alcohol	0.88	0.34	2.8	468
<i>n</i> -Amyl alcohol	0.88	0.34	2.6	495
Allyl alcohol	0.99	0.35	2.8	477
Diffusion in benzene solutions at 7.5°C.				
Methyl iodide	2.06	3.46	0.59	964
Ethyl iodide	1.77	3.39	0.53	903
<i>n</i> -Propyl bromide	1.71	3.55	0.48	908
<i>n</i> -Propyl iodide	1.67	3.36	0.50	908
<i>n</i> -Butyl bromide	1.68	3.48	0.48	948
<i>n</i> -Butyl iodide	1.53	3.31	0.45	875
<i>n</i> -Amyl bromide	1.42	3.42	0.42	836
<i>n</i> -Amyl iodide	1.41	3.28	0.43	846
Octyl bromide	1.17	3.29	0.36	770
Ethylene dichloride	1.77	3.71	0.48	896
Carbon tetrachloride	1.51	3.40	0.43	818
Phenyl bromide	1.45	3.39	0.43	808
Phenyl iodide	1.35	3.27	0.42	768

three-fold in the radius of the diffusing molecule is noted. The constancy of $D\eta$, if equation 68 be applied, depends, however, on the equality of ΔF^\ddagger for viscous flow and for diffusion and on the equality of λ_v and λ_D , the distances between successive minima for the viscous flow process and the diffusion process, respectively. One might expect the quantity $D \frac{\lambda_2 \lambda_3}{\lambda_1}$ to be more nearly constant. It is impossible at present to evaluate $\frac{\lambda_2 \lambda_3}{\lambda_1}$

satisfactorily, but as a crude test we may, for similar molecules, identify this quantity with $(V/N)^{1/3}$, where V is the molecular volume and N is Avogadro's number. In table 15, values of $D(V/N)^{1/3}$ are given (η is

TABLE 16
Diffusion data for one solute in a number of solvents

SOLVENT	ΔE_{vap}	n	$D_{\text{obed.}} \times 10^4$	$D_{\text{calcd.}} \times 10^4$	$D_{\text{obed.}}/D_{\text{calcd.}}$	α	D
Diffusion of bromobenzene in various solvents at 7.5°C.							
Ether .. .	6.16	4	3.50	16.5	0.21		962
Benzene . . .	7.69	3	1.41	3.1	0.45		1113
Toluene . . .	8.5	4	1.59	7.4	0.22		1097
Cyclohexane .	7.59	3	1.16	3.3	0.35		1334
Hexane . . .	7.0	4	2.59	13.3	0.20		932
<i>m</i> -Xylene . .	9.77	4	1.52	4.9	0.31		850
<i>m</i> -Cymene	10.3	4	1.18	4.1	0.29		

Diffusion of bromoform in various solvents at 20°C.

Acetone . . .	7.27	4	2.69	11.9	0.23	0.018	
Ether .. .	6.1	4	3.39	17.3	0.20	0.017	
Benzene . . .	7.45	3	1.69	3.7	0.45	0.024	
Methyl alcohol . .	8.44	3	1.93	2.6	0.74	0.022	
Ethyl alcohol . . .	9.97	3	0.97	1.3	0.74	0.028	
Propyl alcohol . .	9.99	3	0.77	1.2	0.64	0.030	
Amyl alcohol . . .	10.6	3	0.52	0.94	0.55	0.034	

Diffusion of iodine in various solvents at 20°C.

Methylene bromide	8.25	3.5	0.83	3.82	0.22	0.020	1422
Benzene	7.45	3	1.93	2.79	0.50	0.018	1238
Carbon tetrachloride . . .	8.0	3	1.37	2.39	0.59	0.019	1302
Toluene . . .	7.99	4	1.96	9.26	0.21	0.016	1132
Chloroform	7.08	4	2.12	10.1	0.21	0.013	1226
Ethyl acetate	7.78	4	2.15	9.8	0.22	0.014	981
Heptane	7.59	4	2.77	11.4	0.24	0.016	1325
Carbon disulfide	6.60	4	3.12	12.4	0.25	0.012	1171
<i>m</i> -Xylene	8.80	4	1.68	7.2	0.23	0.017	1079
Isoamyl acetate	9.00	4	1.24	6.7	0.19	0.021	1077
Bromobenzene . . .	8.80	3	1.20	1.7	0.71	0.017	1358
Carbon tetrabromide	11.2		.18			0.041	1782
Methyl alcohol	8.44		1.81			0.018	1108
Phenetole			0.97			0.023	1283
Anisole . . .	8.8		1.13			0.024	1219

nearly constant for the dilute solutions in the same solvent). It is seen that these values are somewhat more constant than are those of D (or $D\eta$) for the series of alcohols diffusing in water and for the series of halogen-

substituted products diffusing in benzene. In calculating the values of D given in table 16, Stearn and Eyring use values of n found by Ewell and Eyring (16) to give the correct temperature variation of viscosity for the particular solvent. Those employed are shown in table 16.

It will be noted that the ratio $D_{\text{obsd.}}/D_{\text{calcd.}}$ is greater than 1 for aqueous solutions and less than 1 for non-aqueous solutions. Stearn and Eyring found this very generally true. They explain the variation of this ratio from unity as due at least partly to a factor in equation 120 which has not been considered. For the case of a liquid with structure, such as water, rotation in the normal state will be hindered more strongly than in the activated state. (Cf. Kincaid and Eyring (37)).

Thus in cancelling out all but one term in the partition functions corresponding to the two states, this factor is overlooked. This would lead to a calculated value too low. For liquids without such pronounced structure rotation may, on the other hand, be less hindered in the normal state, and neglect of this factor would then lead to results which are too high.

A. Diffusion in concentrated solutions

The results of Stearn, Irish, and Eyring (61) and of Powell, Roseveare, and Eyring (52) show three kinds of behavior, two of which are adequately described by equation 75. These classes of systems are:

(a) Liquid mixtures which form nearly perfect solutions, e.g., benzene-carbon tetrachloride. For this case $(d \ln a/d \ln N)$ will be constant and $D\eta$ should be linear with composition, owing to change of $\lambda_1/\lambda_2\lambda_3$ with composition (cf. equation 66). This class is exemplified by curves A of figure 20.

(b) Liquid mixtures which do not form perfect solutions but which are such that ΔF^\ddagger for viscous flow is the same as ΔF^\ddagger for diffusion. For such cases $D\eta$ will not, in general, be linear with composition but $D\eta/(d \ln a_1/d \ln N_1)$ should be. This class is exemplified by curves B and C of figure 20, in which the changes of $D\eta$ and of $D\eta/(d \ln a_1/d \ln N_1)$ with composition are shown, respectively, for the systems chloroform-acetone and chloroform-ether.

(c) Liquid mixtures with well-defined structure such that ΔF^\ddagger for viscous flow may not cancel ΔF^\ddagger for diffusion. The behavior of two such systems, water-methyl alcohol and water-ethyl alcohol, is shown in curves D and E of figure 20. This behavior is typical of four such systems investigated, all four showing a maximum in the value of $D\eta/(d \ln a_1/d \ln N_1)$.

B. Relation of diffusion to volume

A further test of the idea that, for diffusion as well as for viscous flow, the energy of activation at constant pressure is in many cases predominantly the energy necessary to provide a hole to diffuse into would be

furnished by the relation between diffusion coefficient and volume. The only available data satisfactory for investigation are those of Cohen and Bruins (8). The same reasoning which explains the linear relationship

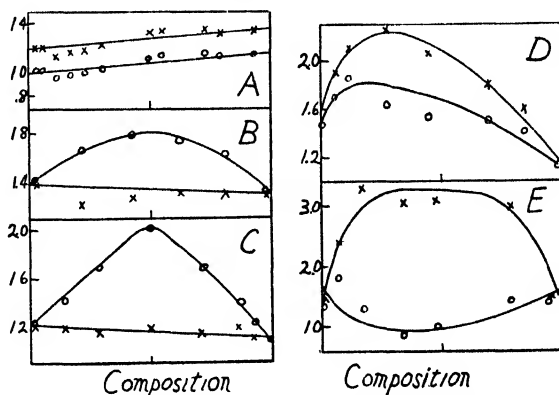


FIG. 20. Plots of composition in mole fraction *versus* $D\eta$ (circles) and *versus* $d \ln a_1 / d \ln N_1$ (crosses) for the complete composition range of several liquid pairs. Curves A are for benzene-carbon tetrachloride, curves B for chloroform-acetone, curves C for chloroform-ether, curves D for water-methyl alcohol, and curves E for water-ethyl alcohol. The ordinate scale for curves E is half that for the other curves.

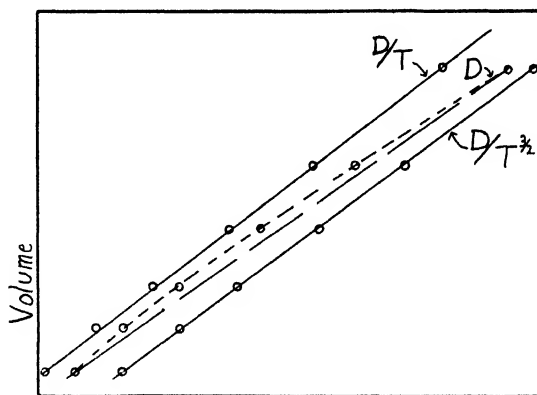


FIG. 21. Plots of volume *versus* D , D/T , and $D/T^{1/2}$, respectively, for the diffusion of tetrabromoethane in tetrachloroethane. The abscissa scales are arbitrary.

between fluidity and volume for certain values of temperature and activation energy leads to a prediction of a linear relationship between volume and either $D/T^{1/2}$ or D/T , depending on whether the partition function for the extra degree of freedom is taken as in equation 119 or that for the solid

(Θ/T) is used. Stearn (60) has used (figure 21) the data of Cohen and Bruins to plot volume against D , D/T , and $D/T^{3/2}$. While the plot of D versus V shows distinct curvature, both the other curves give straight lines.

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TRANSANNULAR PEROXIDES

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Received January 28, 1941

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I. INTRODUCTION

During the years 1912 to 1913 Nelson and Wallach, working independently, made the discovery that ascaridole, the principal constituent of chenopodium oil, is a naturally occurring, liquid, organic peroxide. It is a relatively stable compound, since it can be distilled with steam or *in vacuo*. Ascaridole differed from other peroxides known at the time of its discovery principally in its structure, which was found to contain a peroxide bridge across a six-membered ring in a 1,4-position. For many years ascaridole was regarded as an oddity without an analog in organic chemistry. It was not until about twenty-five years later that other peroxides of similar constitution were discovered. The intensification of the studies on sterols, which began with the discovery of their relation

¹ Fellow of the Jane Coffin Childs Fund for Medical Research.

to vitamin D, led to the observation by Windaus and collaborators that ergosterol and similar sterols are prone to absorb oxygen in the presence of light to form nicely crystalline peroxides. Extensive studies demonstrated convincingly that these peroxides contained a peroxide bridge across ring B of the steroid molecule in a 1,4-position. More recently it was also shown by Bergmann and Skau that an arrangement of double bonds in ring A of the steroid ring system also favored the formation of crystalline peroxides.

The occurrence of such cyclic peroxides is not restricted to alicyclic chemistry. This was first convincingly demonstrated by Dufraisse, who proved that "rubrene peroxide" was actually the peroxide of 5,6,11,12-tetraphenylnaphthacene, and that it carried a peroxide bridge across the ring at C₆ and C₁₁. In a series of investigations, Dufraisse and collaborators established the fact that the formation of such crystalline peroxides is not a peculiarity of rubrene, but that it is typical for the anthracene nucleus. Even anthracene itself can be photooxidized in solution to give the crystalline 9,10-peroxidoanthracene.

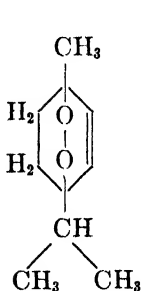
Up to the present time the peroxides mentioned in the preceding paragraph have been regarded as more or less isolated cases, and no attempt has as yet been made to organize and review them under a common denominator. These peroxides have one structural feature in common: a 1,4-peroxide bridge across a six-membered ring. All of them belong to a class of compounds which may be designated as transannular peroxides. The readiness with which many of these peroxides are formed under the influence of light and air, as well as the ease with which some of them can rearrange into more stable compounds or release oxygen, strongly suggest that they may play an important rôle in biological oxidation processes.

II. ASCARIDOLE

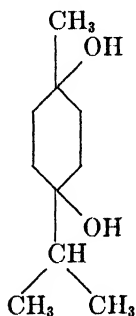
The longest known transannular peroxide is ascaridole, C₁₀H₁₆O₂, which was recognized in 1908 (82) as the principal anthelmintic constituent of chenopodium oil. It is an oily liquid of unpleasant odor and taste. When heated under ordinary pressure to 130–150°C., it undergoes a violent decomposition during which the temperature rises suddenly to 250°C. Combustible gases, consisting chiefly of propane, are evolved, which when ignited can lead to serious explosions. Under a reduced pressure of 4–5 mm., ascaridole distills at 83°C. without decomposition, but not without a certain amount of isomerization. Ascaridole is neutral and is indifferent to all reagents for hydroxyl and carbonyl oxygen. These properties at once indicate the peculiar character of ascaridole, which is without analog in the chemistry of ethereal oils.

The elucidation of the structure of ascaridole was mainly accomplished by Wallach (86) and Nelson (77). Formula I, proposed by Wallach in 1912, best expresses the properties and reactions of this unusual compound.

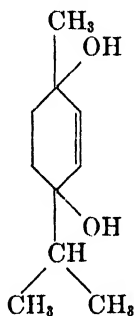
Hydrogenation of ascaridole with colloidal palladium proceeds with unusual rapidity to give *p*-menthane-1,4-diol (II) (86). Under suitable conditions the hydrogenation can be controlled to give dihydro products only. Richter and Presting (79) demonstrated that the mild reduction of ascaridole with palladium saturated with hydrogen gives rise to a mixture of compounds containing 2-*p*-menthene-1,4-diol (III). Paget (78) confirmed this observation but stated that the principal reduction product under such conditions is 1,4-peroxidomenthane (IV), which is also obtained as the sole product of the hydrogenation of ascaridole with a platinum oxide catalyst. This observation is a very surprising one, since it



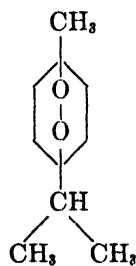
I
Ascaridole



II
p-Menthane-
1,4-diol



III
2-*p*-Menthene-
1,4-diol

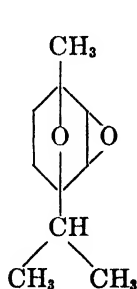


IV
1,4-Peroxido-
menthane
or
dihydroascaridole

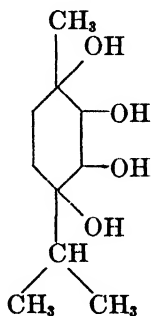
demonstrates that, contrary to all expectations, the double bond is hydrogenated in preference to the peroxide bridge. At present the 1,4-peroxidomenthane (IV) is the only known transannular peroxide of a completely saturated ring system.

When heated in a cymene solution to 150°C., ascaridole isomerizes. One oxygen atom of the peroxide bridge splits off and adds to the double bond to give a dioxide (V) (77, 79, 85). Hydrolysis of the dioxide with dilute sulfuric acid leads to three different products, which are formed by the opening of one or the other or both of the oxide rings. The opening of both rings yields an "erythritol" (VI), the constitution of which was proven by its conversion to the dicarboxylic acid $C_{10}H_{18}O_6$ (VII) and 1,1-dimethyl-acetylacetone (VIII). Hydrolysis of only the ethylene oxide ring gives rise to 1,4-oxido-*p*-menthane-2,3-diol (IX), which yields ascaric acid (X)

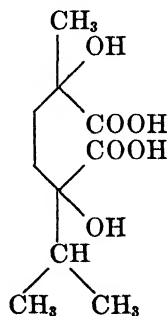
on oxidation. Opening of the 1,4-oxygen bridge leads to the formation of 2,3-oxido-*p*-menthane-1,4-diol (XI). The constitution of this diol



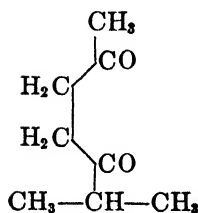
V



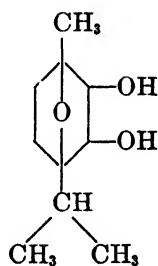
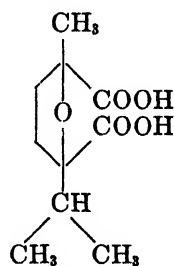
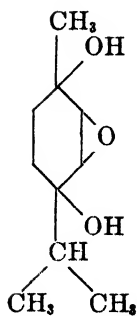
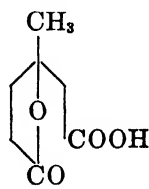
VI



VII



VIII

IX
1,4-Oxido-*p*-menthane-2,3-diolX
Ascaric acidXI
2,3-Oxido-*p*-menthane-1,4-diolXII
1-Methyl-3,4-oxido-1-cyclohexanolXIII
Lactone of β -hydroxy- β -methyladipic acid

was established by its synthesis from 2-*p*-menthene-1,4-diol (III) and perbenzoic acid.

The action of an acid solution of titanous chloride on ascaridole (I) and dihydroascaridole (IV) has recently been studied by Paget (78). Ascaridole reacts with this reagent to give propane and *p*-cresol, each in a yield of about 30 per cent. The evolution of propane had already been observed by Nelson (77) during his attempts to reduce ascaridole with ferrous sulfate. The reduction of dihydroascaridole (IV) yields close to 90 per cent of propane and 1-methyl-3,4-oxido-1-cyclohexanol (XII). The formula for this new oxide is based on the observation that it gives the lactone of β -hydroxy- β -methyladipic acid (XIII) upon oxidation (78).

Ascaridole has been shown by Marvel and collaborators (80) to be an active catalyst for the reaction between sulfur dioxide and olefins of the type $\text{RCH}=\text{CH}_2$, and between sulfur dioxide and acetylenes of the type $\text{RC}\equiv\text{CH}$.

III. PEROXIDES OF STEROIDS

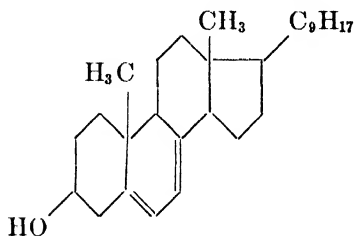
The discovery of the first transannular steroid peroxide was a by-product of the early work on vitamin D. During the years 1927 to 1928 Windaus and collaborators undertook a series of investigations with the object of effecting the conversion of ergosterol into vitamin D by light containing few if any ultraviolet rays. These studies were based on the working hypothesis that the "natural" formation of vitamin D from ergosterol was caused by the action of sunlight of low ultraviolet intensity with the aid of certain naturally occurring sensitizers, such as plant pigments. In the first series of experiments, alcoholic solutions of ergosterol containing small amounts of chlorophyll, carotinoid pigments, and anthocyanidin pigments were exposed in the presence or absence of air to sunlight or the light of a strong electric light bulb. When these investigations failed to give tangible results, the natural pigments were replaced by synthetic sensitizers, such as eosin and erythrosin. It was during this series of experiments that it was discovered that the irradiation of an alcoholic solution of ergosterol in the presence of eosin and air led to the formation of a stable, nicely crystalline compound of melting point 178°C . It contained two more oxygen atoms than ergosterol; since it liberated iodine from a solution of potassium iodide, it was regarded as a peroxide of ergosterol.

The elucidation of the structure of the new peroxide was greatly retarded by a number of conflicting and confusing observations and it was not completed until about ten years after the discovery of the peroxide. In the meantime other steroid peroxides were discovered, the structures of two of which were established without difficulty. These peroxides are dehydroergosterol peroxide and 2,4-cholestadiene peroxide, and have been shown

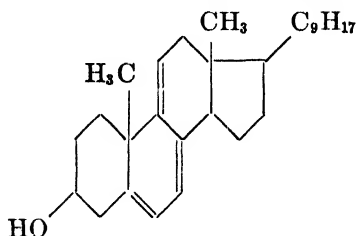
beyond doubt to be transannular peroxides. Since the elucidation of their constitution has been instrumental in the final establishment of the structure of ergosterol peroxide, their discussion will precede that of the latter.

A. Dehydroergosterol peroxide

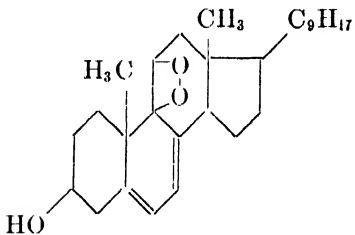
Dehydroergosterol (XV) is readily formed by the dehydrogenation of ergosterol (XIV) with mercuric acetate (97). Like ergosterol, it forms a nicely crystalline peroxide when irradiated in an alcoholic solution in the presence of eosin and oxygen (97). The peroxide shows no selective absorption in the ultraviolet above 230 $m\mu$. This observation excludes from



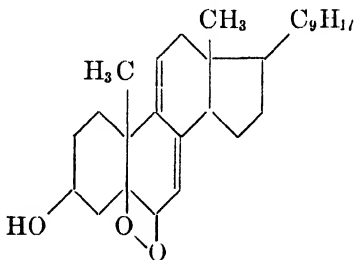
XIV
Ergosterol



XV
Dehydroergosterol



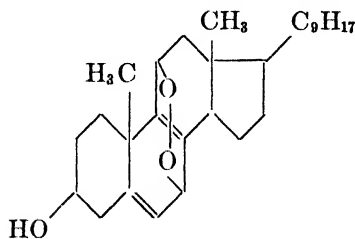
XVI



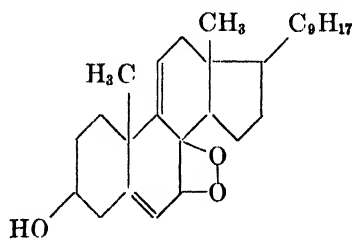
XVII

further consideration all structures containing a system of conjugated double bonds, since they are expected to show selective absorption between 248 and 254 $m\mu$ (76). The structures XVI and XVII for the peroxide are therefore at once eliminated. Of the remaining three possible structures, one (XVIII) can be excluded by considering the results of the catalytic hydrogenation of the peroxide. This leads to the formation of an ergostenediol which can be acetylated to a monoacetate only. This observation proves that the diol contains a tertiary hydroxyl group besides the original hydroxyl group at C₃ (76). A third hydroxyl group must have been eliminated during the hydrogenation. Since it is difficult to visualize how such a tertiary hydroxyl group could have been formed by the hy-

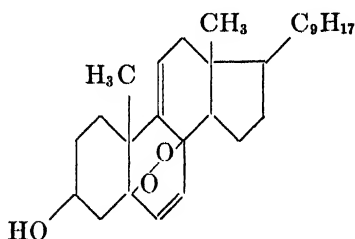
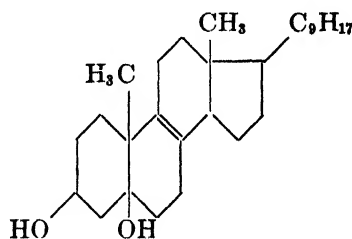
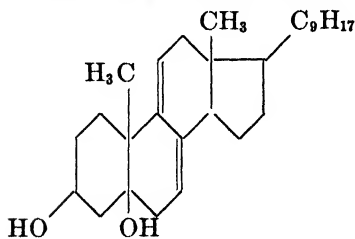
drogenation of a compound of structure XVIII, this structure can be eliminated from further consideration. Other investigations (76) on the constitution of the ergosterenediol proved the presence of the tertiary hydroxyl group at C₅ and hence eliminated formula XIX. Dehydroergosterol peroxide must therefore be a transannular peroxide of the structure XX.



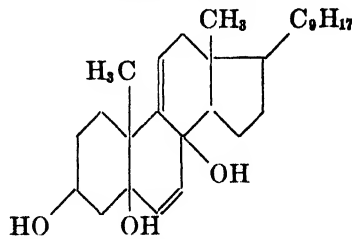
XVIII



XIX

XX
Dehydroergosterol peroxideXXI
Ergosterenediol

XXII



XXIII

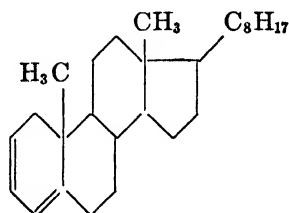
Hydrogenation of this peroxide to ergosterenediol, for which structure XXI has been established, might conceivably go by way of a 3,5,8-triol, which is immediately dehydrated to form a double bond between C₈ and C₉, which is very resistant to further hydrogenation.

Structure XX also finds additional support in the results of the reduction of dehydroergosterol peroxide with zinc in alkali (76, 93). This leads to the formation of a compound with three double bonds, including the

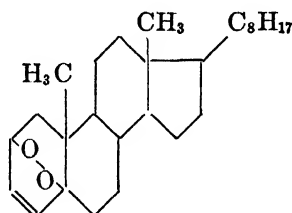
original double bond in the side chain, one secondary hydroxyl group at C₃, and one tertiary hydroxyl group. This trienediol shows selective absorption in the ultraviolet; hence the two ring double bonds must be conjugated. Their conjugation must extend over two rings, since the compound fails to react with maleic anhydride in the normal manner. The properties of this trienediol are best represented by formula XXII. Its formation from dehydroergosterol peroxide has probably proceeded by way of a trienetriol (XXIII), elimination of the hydroxyl group at C₈, and shifting of the ring double bonds into conjugation.

B. 2,4-Cholestadiene peroxide

The dehydration of cholesterol with alumina leads to the formation of 2,4-cholestadiene, which contains two conjugated double bonds in ring A of the steroid ring system (XXIV) (83, 84). Photooxidation of this diene leads to one of two products, depending upon the light source (83). When a 200-watt Mazda bulb is used, a peroxide melting at 113–114°C., is obtained. When, on the other hand, the photooxidation is carried out in the presence of sunlight, an isomeric substance melting at 172°C., is formed; this substance is not a peroxide but a ketone.



XXIV
2,4-Cholestadiene



XXV
2,4-Cholestadiene peroxide

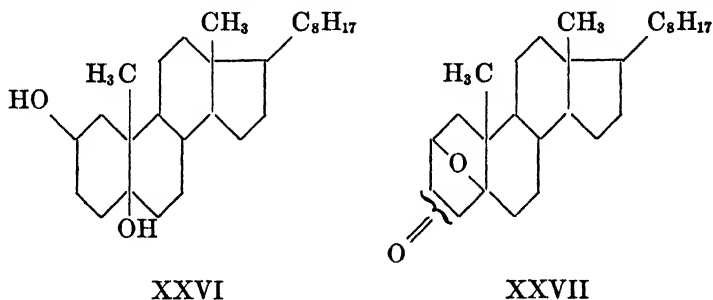
The catalytic hydrogenation of cholestadiene peroxide leads to the formation of a saturated diol, which contains a tertiary hydroxyl group since it gives a monoacetate only, and in which the hydroxyl groups cannot be in vicinal positions, since the diol fails to react with lead tetraacetate. These observations prove structure XXVI for the diol and therefore structure XXV for the peroxide. The alternative formulas for the peroxide, —namely, those of a 2,3- or 4,5-peroxide,—can be excluded from consideration, because substances of such constitution would be expected to give either a diol devoid of a tertiary hydroxyl group or a diol oxidizable with lead tetraacetate.

When irradiated with sunlight, 2,4-cholestadiene peroxide rearranges into the same ketone which is obtained by the photooxidation of 2,4-cholestadiene in sunlight (7, 10). One can therefore assume that the

mechanism of the photooxidation of 2,4-cholestadiene in sunlight involves the formation of the peroxide, followed by its rearrangement into the ketone. It has been suggested that the rearrangement of the peroxide into the ketone is analogous to the isomerization of ascaridole (I) to the dioxide (V). Under the influence of sunlight, one of the oxygen atoms of the peroxide bridge of the 2,4-cholestadiene peroxide probably splits out and adds to the double bond between C₃ and C₄. The ethylene oxide may then rearrange into a C₈- or C₄-ketone (XXVII).

C. Ergosterol peroxide

The convincing proof for the transannular structure of two steroid peroxides made it logical to assume that other steroid peroxides of similar properties are of the same character. For ergosterol peroxide, however, two fundamentally different structural formulas have been proposed. On the basis of their experimental work the group of Göttingen investigators

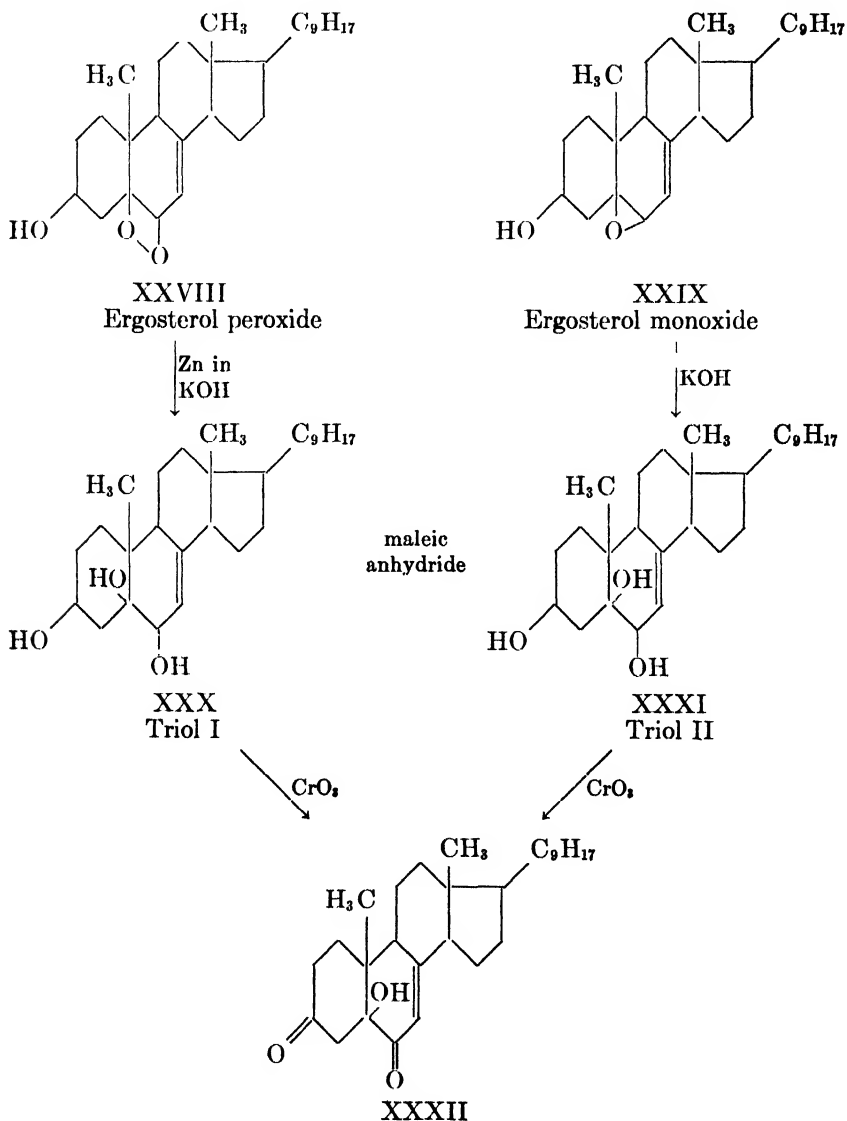


(1, 76), as well as Heilbron (59), have come to the conclusion that the peroxide bridge in ergosterol peroxide is not transannular but is attached to C₅ and C₆ (XXVIII). Fieser (59), on the other hand, has concluded that the available experimental evidence can be better interpreted in favor of a transannular formula for ergosterol peroxide (XXXIII).²

The establishment of formula XXVIII for ergosterol peroxide has been based principally on the study of ergostadienetriol (97), "triol I," which is obtained by the reduction of ergosterol peroxide with zinc in alkali. On heating with maleic anhydride, triol I isomerizes into 3,5,6-trihydroxy-ergostadiene, "triol II" (XXXI). This triol, whose constitution has been well established, is also the product of hydrolysis of ergosterol monoxide (XXIX) (1, 98). The ease of rearrangement of triol I into triol II seemed to prove that the two compounds were not mere position isomers but were stereoisomers of the *cis*- and *trans*-decalin type. They were assigned the structural formulas XXX and XXXI, respectively. Heilbron

² A similar suggestion was made by Luttringhaus (67) in 1931.

(50) gave as additional evidence in favor of such formulations the fact that both triols render the same diketone compound (XXXII) on oxidation.



He reasoned that during the oxidation of triol I (XXX), the hydroxyl group at C₅ had undergone inversion from the *trans*- into the *cis*-position with respect to the methyl group at C₁₀. It seems more logical, however,

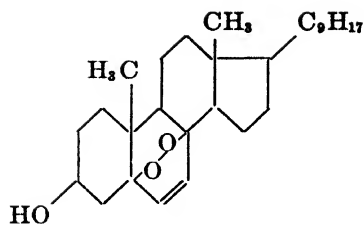
to interpret the fact that both triols give the same diketone compounds as proof for the identical position of the hydroxyl group at C₅ (7).

Since the reactions mentioned above were thought to prove that triols I and II were stereoisomers, the conclusion was drawn that triol I had the formula XXX and that ergosterol peroxide carried a peroxide bridge at C₅ and C₆ (XXVIII). The formulation of triol I as a 3,5,6-trihydroxy compound can not, however, be readily reconciled with several of its properties. On acetylation it gives a monoacetate only (1), and on distillation *in vacuo* it loses two molecules of water to give dehydroergosterol (97) (XV). In contrast, triol II (XXXI) forms a diacetate, and can be distilled without decomposition (98). It was first pointed out by Fieser (59) that these observations strongly indicate the presence of two tertiary hydroxyl groups in triol I and favor its formulation as a 3,5,8-triol (XXXIV), which obviously must have been derived from a transannular peroxide (XXXIII). The transformation of triol I (XXXIV) into triol II (XXXV) is explained by Fieser on the basis of an allylic shift.

Fieser's formulas for ergosterol peroxide (XXXIII) and triol I (XXXIV) are the most logical expressions for all the known properties of these compounds. The fact that triols I and II form the same oxidation product does not contradict Fieser's formula for triol I. It is quite conceivable that, as the first step of oxidation of triol I (XXXIV), a 3-ketotetrahydroxy compound is formed (XXXVI), which loses, first, one molecule of water to give a diketodiol (XXXVII) and then a second molecule of water to give Heilbron's diketone (XXXVIII) (7).

Ergosterol peroxide is surprisingly stable up to 170°C., but at 185–190°C. it undergoes isomerization (67, 94). The isomer, C₂₈H₄₄O₃, lacks all the characteristic properties of a peroxide. Acetylation and reaction with methylmagnesium iodide show the presence of one hydroxyl group (C₃). A second oxygen atom belongs to a keto group, for the isomer gives a monoxime. The third oxygen atom, which is unreactive, is regarded as oxide oxygen.

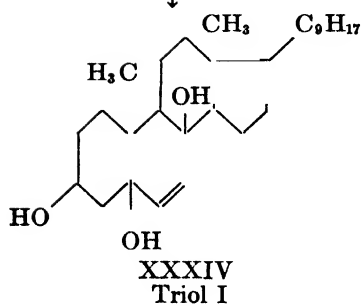
The thermal isomerization of ergosterol peroxide is strongly reminiscent of the thermal rearrangement of ascaridole and the photorearrangement of 2,4-cholestadiene peroxide. As in the case of the isomerization of these two peroxides, the rearrangement of ergosterol peroxide is probably due to a change from a peroxide to an oxide bridge and an addition of oxygen to a double bond to form first an epoxide and then a ketone. Since ergosterol peroxide has two double bonds, the addition of oxygen may take place either at the ring double bond or at the double bond in the side chain to give compounds of the possible structures XXXIX or XL. Because of the failure of the isomer to yield 1,2-dimethylbutyraldehyde (61) upon ozonization, the latter formula is to be preferred.



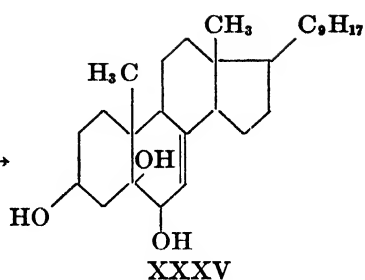
XXXIII

Ergosterol peroxide

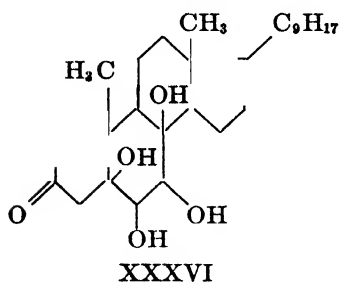
Zn in KOH



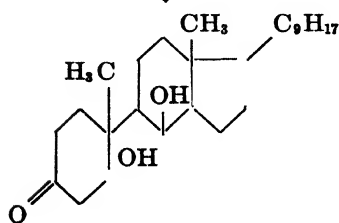
allylic
shift



CrO₃

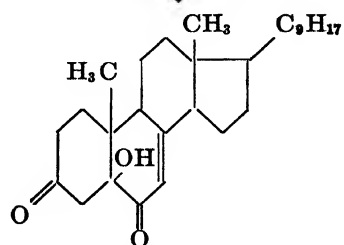


XXXVI

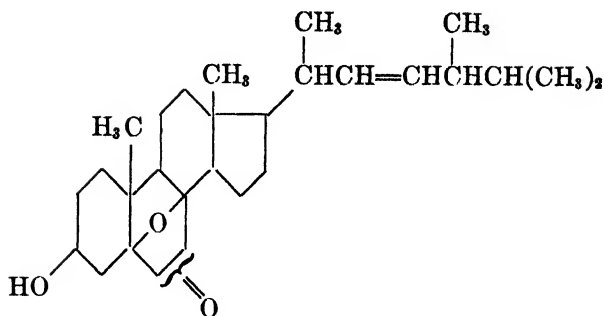


XXXVII

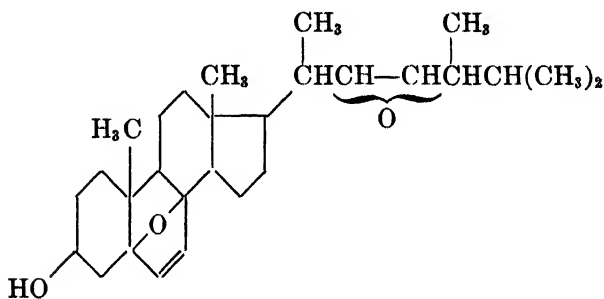
CrO₃



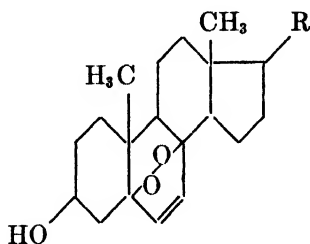
XXXVIII



XXXIX



XL



XLI

(a) $R = C_9H_{19}$ (b) $R = C_8H_{17}$ (c) $R = OH$ *D. Other steroid peroxides*

Three other steroids with a constitution similar to that of ergosterol have given crystalline peroxides upon photooxidation in the presence of a sensitizer. They are the peroxides of 22-dihydroergosterol (96), 7-dehydrocholesterol (81), and 5,7-androstadienediol (11). Although few data have

as yet appeared concerning the constitution of these peroxides, it appears logical to assume that they are of transannular character with a peroxide bridge between C₅ and C₈ (XLI, a to c).

IV. AROMATIC TRANSANNULAR PEROXIDES

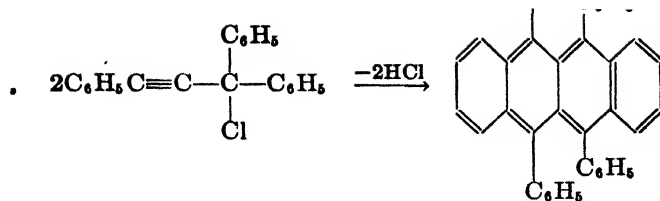
A. Preparation

Most of our present-day knowledge of the preparation, properties, and constitution of aromatic transannular peroxides is based on the results of a series of systematic studies which have been carried out under the direction of Moureu and Dufrasse. These studies began about fifteen years ago with an investigation of the properties of the hydrocarbon rubrene. This red polynuclear substance, C₄₂H₂₈, was first prepared in 1926 (70) by the removal of hydrochloric acid from (phenylethynyl)diphenylmethyl chloride (XLII). Moureu and collaborators (70) observed that solutions of this hydrocarbon rapidly lose their color and fluorescence when exposed to sunlight or artificial light in the presence of air. Concentration of such decolorized solutions leads to the precipitation of a compound of the formula C₄₂H₂₈O₂ plus solvent of crystallization (72) and possessing the properties of a peroxide.

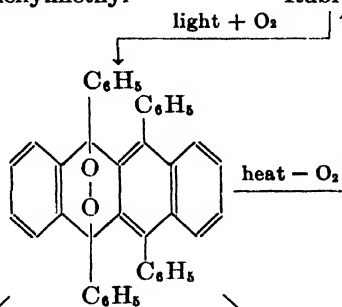
When this compound is heated *in vacuo* a reversal of the reaction takes place; under luminescence (71) the peroxide dissociates into rubrene, solvent of crystallization, and oxygen. The dissociation is not a quantitative one, since not more than 80 per cent of the theoretical amount of oxygen is obtained. The evolution of carbon dioxide during the reaction indicates that approximately 5 per cent of the peroxide undergoes decomposition. Extension of these investigations to a number of derivatives of rubrene, the "rubenes," established the fact that the ability to add oxygen in the presence of light is a general property of this class of compounds.

Because the exact constitution of rubrene remained unknown for a number of years, the structure of rubrene peroxide was not fully established until 1936. By that time evidence had accumulated (17) which proved that rubrene is 5,6,11,12-tetraphenylnaphthacene (XLIII), and that the other hydrocarbons of the rubene series are also derivatives of naphthacene. This new formulation at once suggested that the new peroxides are either 1,2- or transannular (1,4) peroxides. Hydrogenation experiments, which will be discussed later, proved conclusively the transannular character of these peroxides and hence the correctness of formula XLIV for rubrene peroxide.

The discovery that linear polynuclear aromatic hydrocarbons are prone to undergo photooxidation was not entirely new. As early as 1867, Fritzsche (60) made the observation that solutions of naphthacene are rapidly decolorized in the presence of light and air to give a crystalline



XLII
(Phenylethynyl)diphenylmethyl
chloride

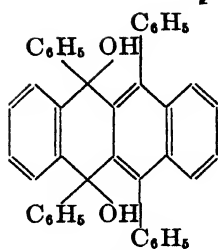


light + O₂

heat - O₂

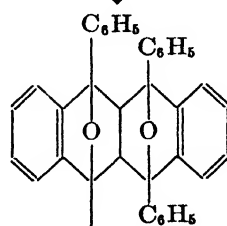
H₂

H₂

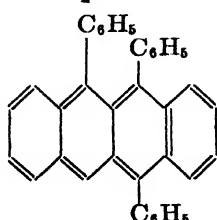


heating

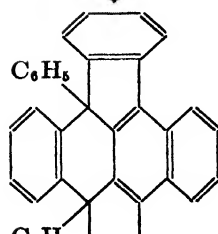
MgI₂



Mg

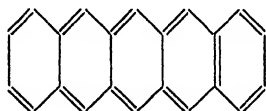


+ C₆H₅OH + MgO

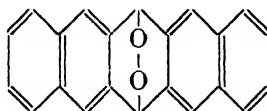


material which regains color on fusion. In the light of the observations of Moureu and Dufraisse, it must be assumed that Fritzsche's compound was naphthacene peroxide. In 1930 Clar and John (13) found that pentacene (L) is so rapidly photooxidized to a peroxide that they recommended that one refrain from working with solutions of this hydrocarbon in broad daylight. This tendency to undergo oxidation is even more pronounced in the case of hexacene (14). The transannular formula for pentacene peroxide (LI) suggested by Clar and John (13) appears to be justified because of its analogy to the structure of naphthacene peroxide.

On the basis of the experience gained during the study of naphthacenes, Dufraisse formulated the hypothesis that the ability of these hydrocarbons to add oxygen is intimately connected with the activity of their meso-positions. Since such meso-positions are also present in the anthracene molecule, Dufraisse decided upon an extension of his investigations to anthracene and its derivatives. He at once made the surprising discovery that not only meso-substituted anthracenes but anthracene (29, 30) itself is readily photooxidized to give a nicely crystalline transannular peroxide



L
Pentacene



LI
Pentacene peroxide

(LII). Dufraisse justifiably prides himself (18) that it was his working hypothesis which led him to the discovery of this interesting reaction. It seems indeed astonishing that anthracene peroxide should have escaped the attention of so many investigators, for the photochemistry of anthracene has been extensively studied in relation to the formation of dianthracene.

Dufraisse's discovery was greatly facilitated by his choice of carbon disulfide as a solvent in which to carry out the photooxidation. The rate of oxidation in this solvent far exceeds that in other solvents which have so far been tested (22). Table 1 shows the comparative rates of oxidation of uniform quantities of rubrene in different solvents. The rates were measured either in terms of time required for complete decolorization of the solutions or in terms of changes in concentration in a given time. The rate of oxidation in benzene was used as standard.

The transannular peroxides of the naphthacene and anthracene series which have so far been prepared are listed in tables 2 and 3. All efforts to bring about the formation of photoperoxides in the phenanthrene, naphthalene (41), or acridine (33) series have been unsuccessful. This

failure again demonstrates that the ability to form photoperoxides is dependent upon the meso additive activity of aromatic hydrocarbons and their derivatives. There exists an intimate relationship between the ability of such compounds to add maleic anhydride and to add oxygen in the presence of light. Polycyclic aromatic compounds which form transannular peroxides also form addition products with maleic anhydride. It can also be assumed that compounds which fail to give the Diels-Alder reaction will also lack the ability to form photoperoxides. 9,9-Dianthryl and 10,10-diphenyl-9,9-dianthryl (49), for example, react neither with maleic anhydride nor with oxygen in the presence of light, a fact which indicates the disappearance or great reduction of the meso activity in these compounds.

TABLE 1

Comparative rates of oxidation of rubrene in various solvents

SOLVENT	COMPARATIVE RATES OF OXIDATION
Carbon disulfide	9
Chloroform	3
Methyl iodide	1
Benzene	1
Acetone	1
Ethyl ether	0.5
Anisole	0.4
Pyridine	0.25
Nitrobenzene...	0.1
75% CS ₂ + 25% ether	2
50% CS ₂ + 50% ether.....	1

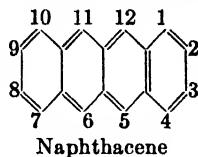
B. Photooxidation of carcinogenic hydrocarbons

Since the most important carcinogenic hydrocarbons are substituted anthracenes, it is of considerable interest to know whether they also can undergo photooxidation to form peroxides. It has been known for some time that solutions of a number of carcinogenic hydrocarbons are photosensitive. Moreover, it has been observed by Boyland (9) that alkaline extracts of such hydrocarbons which had been exposed to light and air arrest the activity of certain enzymes. It has also been found by Maisin and de Jonghe (68) that light accelerates the production of skin tumors in mice treated with 3,4-benzopyrene.

Independent investigations by Velluz (99), Cook (12), and the authors of this review (8) failed to find a way for the preparation of the peroxides of 1,2,5,6-dibenzanthracene and methylcholanthrene. In order to ascertain whether a small amount of peroxide is formed which might escape

isolation, Velluz (99) irradiated the two hydrocarbons, dissolved in carbon disulfide, in an apparatus permitting measurements of the oxygen uptake. The reluctance of these hydrocarbons to add oxygen was indicated by the fact that no appreciable absorption had taken place even after two weeks of irradiation. In the case of 1,2-benzanthracene (12), 9,10-diphenyl-1,2-

TABLE 2
Transannular-(6,11)-peroxides of naphthacene and derivatives



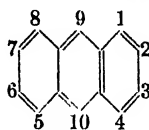
PEROXIDE OF	DISSOCIA- TION IN PER CENT OF OXYGEN	REFER- ENCES
Naphthacene	0	(31)
5,11-Diphenylnaphthacene	0	(39)
6,11-Diphenylnaphthacene	0	(32)
5,6,11-Triphenylnaphthacene	15	(21)
5,6,11,12-Tetraphenylnaphthacene (rubrene)	80	(70)
5,11-Di(<i>p</i> -tolyl)-6,12-diphenylnaphthacene*	70	(40)
5,6,11,12-Tetraphenyl-2,8-dimethylnaphthacene*	66	(37)
11-(<i>p</i> -Tolyl)-5,6,12-triphenyl-2-methylnaphthacene	64	(37)
5,11-Di(<i>p</i> -tolyl)-6,12-diphenyl-2,8-dimethylnaphthacene	74	(40)
5,11-Di(β -naphthyl)-6,12-diphenylnaphthacene	80	(76)
5,11-Di(<i>p</i> -bromophenyl)-6,12-diphenylnaphthacene*	69	(24)
5,11-Di(<i>p</i> -bromophenyl)-6,12-diphenyl-2,8-dibromo- naphthacene	50	(43)
5,11,6,12-Tetra-(<i>p</i> -bromophenyl)-2,8-dibromonaphthacene	76	(44)
5,11-Di(<i>p</i> -methoxyphenyl)-6,12-diphenylnaphthacene	52	(23)
5,11-Di(<i>p</i> -carboxyphenyl)-6,12-diphenylnaphthacene	59	(25)
5,11-Diphenyl-6,12-di(biphenyl)naphthacene	70	(52)
2,6,8,12-Tetraphenyl-5,11-di(biphenyl)naphthacene	70	(51)
1,2,3,4-Tetrahydro-6,11-diphenylnaphthacene	80	(32)
5,6,12-Triphenyl-11-carbethoxynaphthacene	44	(6)
5,11-Diphenyl-6,12-dicarbethoxynaphthacene	15	(38)

* These peroxides undergo isomerization (see page 387).

benzanthracene (101), and 1,2,3,4-tetrahydro-9,10-diphenyl-1,2-benzanthracene (101), indications of the formation of peroxides were found, but contaminations hindered their isolation. Better results were obtained with meso-dimethyl-substituted hydrocarbons. One of the most rapidly acting carcinogenic hydrocarbons now known is 9,10-dimethyl-1,2-benz-

anthracene. Since Bachmann (5) had shown that this hydrocarbon possesses a surprisingly high meso additive activity toward maleic an-

TABLE 3
Transannular-(9,10)-peroxides of anthracene and derivatives



Anthracene

PEROXIDE OF	DISSOCIA- TION IN PER CENT OF OXYGEN	REFERENCES
Anthracene	0	(29, 30)
9-Methylantracene	0	(91)
9-Ethylantracene	0	(91)
9,10-Dimethylantracene	0	(91)
9-Methyl-10-ethylantracene	0	(91)
9-Cyclohexylantracene	0	(92)
9-Phenylantracene	12	(48)
9-Phenyl-10-methylantracene	20	(90)
9-Phenyl-10-ethylantracene	35	(90)
9-Phenyl-10-cyclohexylantracene	48	(92)
9,10-Diphenylantracene	96	(27, 36)
9,10-Di(o-tolyl)anthracene (impure)	83	(89)
9,10-Di(m-tolyl)anthracene	94	(89)
9,10-Di(p-tolyl)anthracene	94	(89)
9,10-Di- α -naphthylantracene	90	(88)
9,10-Di- β -naphthylantracene	95	(88)
9,10-Diphenyl-2-bromoanthracene	91	(100)
9,10-Diphenyl-2-carboxyanthracene	91	(100)
9,10-Diphenyl-2-carbomethoxyanthracene	92	(100)
9,10-Diphenyl-1,4-dimethoxyanthracene	98	(47)
9-Phenyl-10-carbomethoxyanthracene	60	(48)
9,10-Dimethoxyanthracene	0	(42, 46)
1,2-Benzanthracene (impure)		(12)
9,10-Diphenyl-1,2-benzanthracene (impure)		(101)
9,10-Diphenyl-1,2,3,4-tetrahydro-1,2-benzanthracene (impure)		(101)
9,10-Dimethyl-1,2-benzanthracene		(12)
5,9,10-Trimethyl-1,2-benzanthracene		(12)
6,9,10-Trimethyl-1,2-benzanthracene		(12)
5,6,9,10-Tetramethyl-1,2-benzanthracene		(12)
9,10-Dimethyl-1,2,5,6-dibenzanthracene		(12)

hydride, Cook (12) felt that its photoperoxide might be more readily formed. This indeed proved to be the case, and equally satisfactory

results were obtained with 5,9,10-trimethyl-, 6,9,10-trimethyl-, and 5,6,9,10-tetramethyl-1,2-benzanthracene and 9,10-dimethyl-1,2,5,6-dibenzanthracene. The fact that such highly carcinogenic hydrocarbons are readily photooxidized makes untenable the assumption of Velluz (99) that the carcinogenic activity of dibenzanthracene and of methylcholanthrene is in some way related to their ability to resist photooxidation.⁸

C. Photooxidation of various derivatives of anthracene

Derivatives of anthracene carrying one halogen atom in a meso-position as, for example, 10-bromo- and 10-iodo-9-phenylanthracene, undergo photooxidation (48). During the reaction, however, some halogen splits off and the peroxides can not be isolated in a pure form. Photooxidation of 9,10-dihydroxyanthracene does not lead to the formation of a crystalline peroxide (42). The disodium salt of this compound, as was already shown by Manchot (69), autoxidizes rapidly even in the dark. Dufraisse (42) was unable to detect the presence of a peroxide during the course of this reaction. The peroxide of 9,10-dimethoxyanthracene has been made, although considerable difficulties were encountered in its preparation (42). It melts with the formation of anthraquinone, but can be sublimed *in vacuo* at 80°C. In the presence of light and air, it is rapidly oxidized to anthraquinone.

Photooxidation of 5,11-di(*p*-carboxyphenyl)-6,12-diphenylnaphthacene gives a peroxide whose salts are water-soluble (25).

D. Reactions of the peroxides

(1) *Reduction*.--Upon catalytic hydrogenation the peroxides add 1 mole of hydrogen to yield the corresponding meso-dihydroxy derivatives. Thus the peroxides of anthracene, 9,10-diphenylanthracene, and rubrene give 9,10-dihydro-9,10-dihydroxyanthracene, 9,10-diphenyl-9,10-dihydroxyanthracene, and 6,11-dihydroxy-5,6,11,12-tetraphenylnaphthacene (XIV) (34). The first two hydroxides have been known for some time; the last one has more recently been prepared by the action of phenyl-

⁸ *Note added in proof*: Since this paper was submitted an article has come to the attention of the reviewers (Cook, J. W., and Martin, R. H.: *J. Chem. Soc.* **1940**, 1125) which contains a more detailed discussion of the peroxides of the 1,2-benzanthracene series. Cook and Martin found that an ordinary gas-filled 200-watt lamp furnishes a convenient source for the facile photooxidation of derivatives of 1,2-benzanthracene. They also demonstrated that derivatives with only one meso-substituent (9-methyl-, 10-methyl-, and 10-isopropyl-1,2-benzanthracene) give peroxides, although less readily than the 9,10-disubstituted compounds. The peroxides are not carcinogenic, for tumors did not result from injection into mice of suspensions in sesame oil of the pure peroxide of the highly carcinogenic 9,10-dimethyl-1,2-benzanthracene.

lithium on 6,11-diphenyl-5,12-naphthacenequinone (3) and by the action of potassium permanganate on rubrene (20). The formation of the meso dihydroxides proves conclusively the transannular nature of the peroxides.

Upon moderate heating the dihydroxide (XLV) loses one molecule (20) of water to give an oxide of the probable structure XVI. The same oxide is also formed by the reduction of the peroxide XLIV (16, 74) with zinc in glacial acetic acid. The oxide is a stable compound which melts without decomposition. Upon reduction it yields rubrene and upon oxidation it forms *o*-dibenzoylbenzene.

Dehydration of the dihydroxide under the influence of strong mineral acids leads (16) to the loss of two molecules of water and cyclization to a hydrocarbon of the probable structure XLVIII.

(2) *Isomerization*.—Under the influence of certain reagents, such as magnesium iodide and strong mineral acids, peroxides of the naphthacene series undergo rearrangement. Addition of magnesium iodide (16, 19) to an ethereal solution of rubrene peroxide brings about an exothermic isomerization which is complete in about 5 min. The isomer no longer gives the reaction of a peroxide. It does not lose oxygen when heated; in fact, it can be distilled *in vacuo* without decomposition. Since it reacts neither with carbonyl nor with hydroxyl reagents, Dufraisse (16) regards it as a transannular 5,12,6,11-dioxide of the structure XLVII. This rearrangement of a peroxide into a dioxide is strongly reminiscent of the isomerization of ascaridole. The peroxides marked with asterisks in table 2 undergo analogous isomerization (53, 54, 57, 58).

The dioxide (XLVII) reacts with Grignard reagents (16) to give a variety of compounds, from which the monoxide (XLVI), the dihydroxide (XLV), and rubrene have been isolated. In the presence of an excess of metallic magnesium, one phenyl group is eventually split off and 5,6,12-triphenylnaphthacene (XLIX) and phenol are obtained, the latter in an almost quantitative yield (21).

When treated with strong acids, rubrene peroxide (54) undergoes a different rearrangement, which as yet is poorly understood. The new isomer melts without decomposition and loss of oxygen and shows the presence of one hydroxyl group. Treatment with zinc in glacial acetic acid transforms this isomer into the polynuclear hydrocarbon of structure XLVIII.

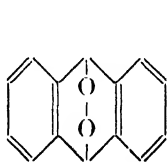
All attempts to rearrange the peroxide of 9,10-diphenylanthracene in an analogous manner have so far been unsuccessful (36). This failure may be due to the absence of a suitable "receiver" for one of the oxygen atoms in the anthracene molecule or to the instability of a transannular oxide linkage in the anthracene molecule. It has not yet been possible (21) to prepare 9,10-diphenyl-9,10-oxidoanthracene, either by partial

reduction of the corresponding peroxide, by mild dehydration of the corresponding dihydroxy compound, or by oxidation of 9,10-diphenylanthracene.

(3) *Reactions with acids*.—Anthracene peroxide (LII) reacts (30) with hydrochloric acid to give chloroanthrone (LIII) and with hydrobromic acid to give bromoanthrone or 9,10-dibromoanthracene, depending on the concentration of the acid.

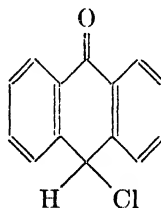
E. Dissociation of peroxides

Moureu and Dufraisse consider as one of the outstanding properties of rubrene peroxides, as well as of analogous compounds, their ability to dissociate on heating into oxygen and the parent hydrocarbon. In the case of rubrene peroxide, this dissociation is already noticeable at room temperature (73). Numerous quantitative studies on the thermal decomposition of peroxides have been carried out by the French investigators, using the following technique: A weighed amount of the peroxide is placed in a



LII

Anthracene peroxide



LIII

Chloroanthrone

glass tube connected with a U-tube, a gas-measuring device, and a vacuum pump. After evacuation of the apparatus at room temperature, the U-tube is cooled to about $-70^{\circ}\text{C}.$, and the peroxide gently heated. The U-tube serves to condense the solvent of crystallization which is present in many peroxides. After the completion of the reaction at $220\text{--}250^{\circ}\text{C}.$, the collected gas is analyzed for oxygen and carbon dioxide. Application of this method to almost all peroxides prepared by the French investigators brought out the fact that there exist marked differences in dissociability. The amounts of oxygen recovered from the various peroxides have been recorded in tables 2 and 3.

The data demonstrate that the dissociability of the peroxides is greatly influenced by the nature of the substituents in the meso-positions. Only the peroxides of 9,10-diaryl-substituted anthracenes yield an almost quantitative amount of oxygen. The dissociability of 9,10-diphenyl-1,4-dimethoxyanthracene peroxide is of unusual magnitude (47). At room temperature this compound dissociates to the extent of 25 per cent in 10

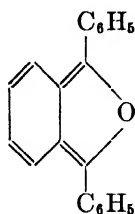
days, 55 per cent in 30 days, and 78 per cent in 40 days. Furthermore, its quantitative dissociation takes place in a few minutes at 80°C., in contrast to many other peroxides whose dissociation is barely perceptible below 100°C. Replacement of one of the aryl groups by an alkyl group or hydrogen greatly reduces the dissociability of the peroxides, and replacement of both groups leads to peroxides showing no recognizable dissociation. In all probability, complete thermal dissociation takes place in all instances. In the case of peroxides with hydrogen or alkyl substituents in the meso-position, however, this dissociation is not detectable by the technique employed, because the liberated oxygen is at once consumed in the oxidation of the molecule. Thus anthracene peroxide on heating does not decompose to give anthracene and oxygen but yields a considerable amount of anthraquinone.

Thermal dissociation of 5,6,11,12-tetraaryl-substituted naphthacene peroxides gives from 60 to 80 per cent of the theoretical amount of oxygen. This yield is low when compared with that of meso-diaryl-substituted anthracene peroxides. The reviewers believe that this is due to a partial rearrangement of the peroxide into a non-dissociable dioxide (XVIII), an isomerization which fails to take place in the case of anthracene peroxides.

Dufraisse (16, 18) has offered elaborate discussions concerning the thermal dissociation of the peroxides and its biological implications. In the opinion of the reviewers, however, the importance of this reaction is overrated. As has been stated several times in this review, there exists an intimate parallel between the addition of maleic anhydride and the photo-addition of oxygen to polycyclic hydrocarbons. Bachmann (4) has disclosed that the addition of maleic anhydride to hydrocarbons containing the anthracene nucleus is a reversible reaction. He found, for example, that the heating of a mixture of equimolecular proportions of maleic anhydride and 3-methylcholanthrene in boiling xylene led to the formation of an adduct in a yield of 22 per cent. The identical equilibrium mixture of hydrocarbon, maleic anhydride, and adduct was obtained by heating a xylene solution of the pure adduct. It seems to the reviewers that the application of similar quantitative methods to the study of the photo-oxidation of hydrocarbons containing an anthracene nucleus is very desirable. It would be more advantageous to study the reversibility of the peroxides on the basis of equilibrium mixtures than on the basis of a thermal decomposition which does not give very illuminating results. The fact that the photooxidation of a number of hydrocarbons does not go beyond a certain yield of peroxide strongly indicates the existence of equilibrium mixtures.

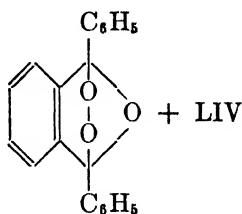
F. Photooxidation of 1,3-diphenylisobenzofuran

It has long been known that 1,3-diphenylisobenzofuran (LIV) is readily photooxidized to *o*-dibenzoylbenzene (LVII). Since the furan possesses an *o*-quinonoid system and adds maleic anhydride readily in the 1,3-position (2, 87), it might be expected that the first product of photooxidation is a transannular peroxide (LV). All attempts, however, have so far failed, either to isolate such a peroxide or to prove its presence. It is conceivable that the peroxide, immediately after its formation, gives off one atom of oxygen with the formation of an oxide bridge (LVI), and that the oxygen is added to a molecule of 1,3-diphenylisobenzofuran to form

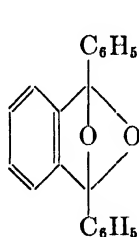


LIV

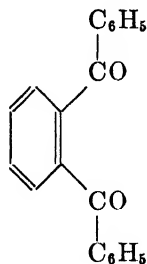
1,3-Diphenylisobenzofuran



LV



LVI



LVII

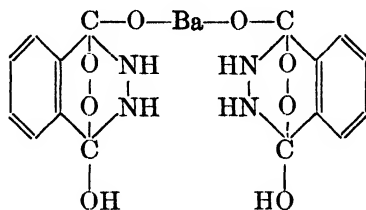
o-Dibenzoylbenzene

a second molecule of the dioxide. The dioxide would then rearrange into *o*-dibenzoylbenzene. This hypothesis receives support from the observation that the oxidation of 1,3-diphenylisobenzofuran to *o*-dibenzoylbenzene can be carried out with one mole of perbenzoic acid (8).

G. Peroxides of doubtful structure

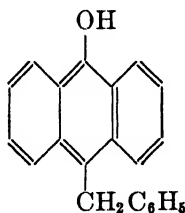
Apart from the peroxides mentioned in the preceding paragraphs, other peroxides of supposed transannular structure are now and then mentioned in the literature. These peroxides have been made almost exclusively by methods other than photooxidation, and the transannular structure is not

based on convincing evidence. Thus Drew and Garwood (15) have proposed the provisional structure LVIII for the barium salt of the peroxide of phthalaz-1,4-dione. Julian and collaborators (65, 66) observed that the passage of oxygen through a moist ethereal solution of 10-benzylanthranol (LIX) leads to the formation of a compound which they believe to be a transannular peroxide (LX). Heating of the compound resulted in the formation of anthraquinone, benzaldehyde, and benzyl alcohol. The reviewers hold, however, that the available evidence is not sufficient to prove beyond doubt the transannular character of the oxidation product of 10-benzylanthracene.



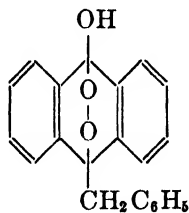
LVIII

Barium salt of phthalaz-1,4-dione



LIX

10-Benzylanthranol



LX

V. CONCLUSION

As has been shown in the preceding paragraphs, there exist a number of organic compounds which are endowed with the ability to fix oxygen in the presence of light to form peroxides of often surprising stability. In all instances the addition takes place across a ring in a 1,4-position to give transannular peroxides. In the case of ascaridole, it may be assumed that this peroxide is the result of the addition of oxygen to α -terpinene. All substances which have been found to give transannular photoperoxides possess a system of conjugated double bonds in one ring; hence they absorb ultraviolet or visible light and add maleic anhydride.

Many of the peroxides which have been described undergo rearrangement under the influence of either heat or light to give a variety of products.

The first step in this rearrangement consists in a change from the peroxide to an oxide bridge and an addition of the "free" oxygen atom to a suitable receiver, such as a double bond in the molecule. The oxides may then be rearranged to ketones. It appears probable that a number of biological oxidations may proceed by way of addition of oxygen to a cyclic diene system and subsequent rearrangement of the ensuing transannular peroxides. The formation of numerous alcohols and ketones of the terpene series from terpene hydrocarbons may be readily conceived in such a manner. In closing, it may be pointed out that a number of compounds which can be photooxidized to give transannular peroxides also give a definite light reaction in the absence of air. Thus anthracene and 1,3-diphenylisobenzofuran are dimerized by light, and ergosterol and similar steroids are dehydrogenated to give compounds with two steroid ring systems attached to each other (63).

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THE PROPERTIES AND FUNCTIONS OF THE PLASMA PROTEINS, WITH A CONSIDERATION OF THE METHODS FOR THEIR SEPARATION AND PURIFICATION¹

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Received March 5, 1941

I. INTRODUCTION

The characterization of blood as a part of the "milieu intérieur" was developed by the great nineteenth-century French physiologist, Claude Bernard. The circulating fluid of the body constituted, from his point of view, the environment of the other tissues. Its functions are now associated with the transport of oxygen as well as of the substances necessary as elements of tissue structure; with the removal of carbon dioxide and waste products; with the balance of water and electrolytes; and with the hormonal control of bodily processes.

Succeeding generations of physiologists have added greatly to our knowledge regarding blood. Meanwhile, the phenomena of immunity were discovered. These phenomena have been correlated on the one hand with resistance to infectious diseases and on the other with changes in the composition of the blood, involving both its cellular and its extracellular constituents. The microscope reveals blood cells of various kinds, among them white cells, or leucocytes, associated by Metchnikoff with phagocytic activity, and red cells, or erythrocytes, which contain hemoglobin, the protein of the blood which, by virtue of its complex prosthetic, iron-containing group, carries oxygen to the tissues. Besides hemoglobin, the red blood corpuscles contain a number of other proteins present in much smaller amount and performing quite different functions, many of them

¹ Delivered before the Chapter of Sigma Xi of Brown University, in Providence, Rhode Island, February 10, 1941.

² So many of my colleagues in this department have contributed to our knowledge of the plasma proteins that this report reflects the activities and thoughts of all of us. Dr. J. L. Oncley has made most of the ultracentrifugal analyses and Drs. S. H. Armstrong, Jr., J. M. Newell, and J. A. Luetscher, Jr., most of the electrophoretic analyses that are reported on the fractions purified by them or by Drs. T. L. McMeekin, J. D. Ferry, L. Pillemer, W. L. Hughes, L. E. Strong, R. M. Ferry, A. A. Green, Mrs. M. H. Blanchard, and Mr. J. H. Weare.

enzymatic. Among the latter are such enzymes as a phosphatase and a carbonic anhydrase. These and other constituents of blood cells may be considered as parts of a tissue circulating in and in equilibrium with the non-cellular constituents of the blood. Many of the substances concerned with the physiological and the immunological functions of the blood are among its extracellular constituents and are also protein in nature.

The cells of the blood are readily separated from the rest of this tissue by permitting the blood to clot. The proteins involved in this complex coagulation phenomenon include prothrombin and fibrinogen. The retreating clot yields most of the other plasma proteins in an amber-colored, transparent, aqueous serum. Provided the blood is withdrawn with care and citrate or oxalate added, clotting is prevented. The cells may then be separated from the blood by sedimentation or centrifugation, yielding a clear, transparent fluid, the plasma.

The main constituent of plasma, as of serum and most other tissues, is water. Each liter of plasma contains over 900 cc. of water.³ By far the most copious constituents of plasma, other than water, are the proteins. These nitrogenous molecules are of such large molecular size that, under normal conditions, they do not pass through the walls of cells which are freely permeable to water, electrolytes, and smaller organic molecules. They are diverse in form and function. Some, as we have seen, are concerned with the clotting of the blood; certain others with immunity from disease. Some are primarily concerned in maintaining the osmotic pressure and thus the water balance of the body; some have pronounced amphoteric or dielectric properties. Some are hormones, others are enzymes, and the functions of many remain to be discovered. The last quarter-century has been marked by great advances in the chemistry of the proteins. It is our purpose in this discussion to consider the plasma as a system with many protein components and to explore, in the light of recent advances, both the chemical nature and the physiological functions of the protein components.

II. THE FIBRINOGEN COMPONENT OF PLASMA

The protein present in plasma varies somewhat in amount from species to species. Thus normal human plasma contains between 6 and 7 per cent protein, and fibrinogen is present to the extent of approximately 6 per cent of the total protein (table 1) (24, 41). The amount of this protein varies, like that of other proteins, not only from species to species, but also in certain diseases (72, 2, 44, 41, 40). The observation that there is wide

³ In the drying of frozen plasma, a large part of the cost of the process depends upon the work necessary to freeze and evaporate this much water for every 60 or 70 g. of protein.

variation in a protein constituent of the plasma suggests that we should consider carefully on the one hand the chemical nature of the molecule, and, on the other hand, the function that it serves.

Double refraction of flow and the shapes of molecules

Among the characteristics of the fibrinogen molecule is its long, rod-like shape. Its molecular weight is not especially large, being of the same order as that of serum globulins. Fibrinogen reveals double refraction of flow (3). That is to say, its molecules are asymmetric and are oriented in a stream flowing with a sufficient velocity, precisely as are the logs in a fast flowing stream (54, 48). This streaming birefringence, in so far as it does not vanish when the refractive index of the medium is equal to that of the molecule, indicates that fibrinogen has a microcrystalline structure and that its molecules are many times longer than they are broad. If the fibrinogen of the blood may be compared to the oriented logs of a fast flowing stream, the clot may be thought of as a log jam.

Solubility and the "salting out" of proteins

One of the conventional methods of separating the proteins from plasma and from each other is by their fractional precipitation with neutral salts. The precipitation of proteins by neutral salts has been employed ever since the middle of the last century (59, 78, 1). "Salting out" depends upon the character of the neutral salt as well as of the protein (71, 29, 16, 19, 22, 53, 28, 30). Thus ammonium sulfate, when added in sufficient amount, will bring about precipitation of essentially all plasma proteins (4, 69). Sodium chloride will precipitate only a few, but fibrinogen is readily "salted out" by sodium chloride and is under most circumstances the first protein to be separated from plasma by salt or by most other protein precipitants.

The solubility of fibrinogen or of any other purified protein in concentrated salt solution is given by the relation

$$\log S = \beta - K_s \mu \quad (1)$$

where S is the solubility of the protein and μ the ionic strength of the solution (5). In figure 1 the logarithm of the solubility of fibrinogen in solutions of various salts is plotted as ordinate and the ionic strength as abscissa. The slope of each curve, K_s , is a function of the protein and the neutral salt and is independent over wide ranges of pH and temperature. Among neutral salts those with monovalent cations and polyvalent anions precipitate proteins at lower concentrations. Ammonium sulfate and potassium phosphate solutions are conveniently used, the latter having the property of simultaneously controlling the acidity or pH (6, 20).

¹ Whereas the slope, K_s , appears to be independent of pH and of tempera-

ture over wide ranges, the constant β defines the change in solubility with change in temperature or in pH (5). In general, it is found that the solubility is minimal when the protein is in an isoelectric condition or at a

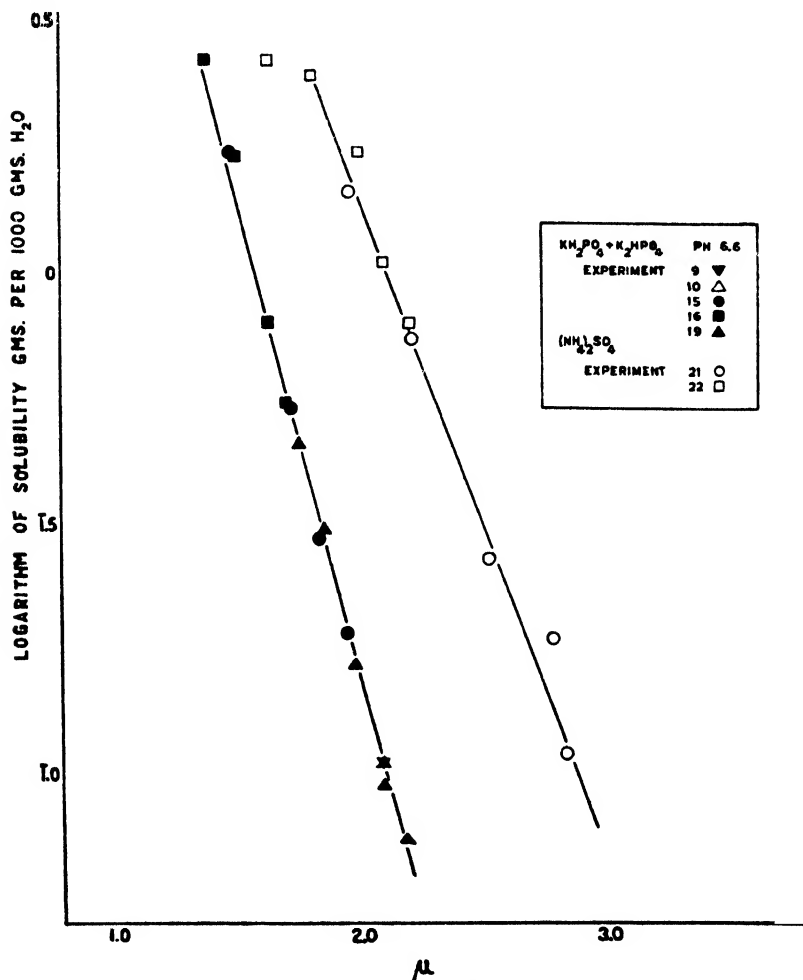


FIG. 1. Solubility of fibrinogen in concentrated phosphate and sulfate solutions at varying ionic strength. From Florkin (16).

reaction somewhat acid to the isoelectric point. This is true both in the absence and in the presence of salt. Separations between proteins can thus often be effected by neutral salt precipitation if they differ sufficiently (a) in K_s , (b) in β , (c) in the dependence of β upon temperature, or (d) in

the dependence of β upon pH. Of these, (c) is related to the heat of solution and (d) to the isoelectric point of the protein.

The alcohol precipitation of proteins at low temperature

Fibrinogen is also the first protein to be precipitated from serum or plasma by such organic solvents as acetone or the alcohols. That these conventional organic solvents should be protein precipitants depends upon the strongly polar nature of most proteins. Not only do acetone, alcohols, and many other organic solvents of low dielectric constant precipitate proteins, but when this operation is carried out at ordinary temperatures they bring about changes in the molecule of such a kind that their solubility in water all but vanishes, and the protein is said to be denatured, much as it is by high temperature.

If alcohol precipitation is carried out at sufficiently low temperatures (close to $-5^{\circ}\text{C}.$), denaturation is often prevented, as was suggested by the earlier studies of Mellanby (49), of Hardy and Gardiner (26), and of many subsequent investigators (13, 80, 38), some concerned with the preparation of antibodies. Thus even egg albumin, a readily denatured protein, has been maintained in ethanol-water mixtures at $-5^{\circ}\text{C}.$ for protracted periods of time, the ethanol removed before the temperature was raised, and the unmodified protein recrystallized (15). Provided denaturation is prevented, alcohol precipitation methods can be substituted for salt precipitation methods. The same dependence of solubility upon pH obtains.⁴ Alcohol precipitation methods, although more likely to denature labile proteins, have the decided advantage that precipitates from ethanol-water mixtures at low temperatures can be readily dried under a vacuum. This yields solid, salt-free, water-soluble, purified proteins which can be prepared in any desired amount by the large-scale methods of industry.

III. THE γ -GLOBULIN COMPONENTS OF PLASMA

If the concentration of salt or of ethanol is further increased after the removal of the precipitated fibrinogen, there separates a fraction of the plasma proteins which has been called in the recent literature the γ -globulin. This characterization is based upon the method of electrophoresis perfected by Tiselius (75).

Electrophoretic mobility

The observation that proteins move in an electric field was first made toward the end of the last century (64). The late Sir William Hardy (25) recognized the significance of the phenomenon as due to the amphoteric

⁴ The dependence upon temperature is, however, quite different and is discussed in the last section of this paper.

properties of proteins, and he and subsequent workers,—notable among whom were Pauli (61, 62, 37) and Michaelis (52, 51),—studied a variety of proteins. Whereas electrophoresis in the hands of workers before Tiselius revealed the isoelectric point and the magnitude of the electric charge borne by the protein at varying acid and alkaline reactions, the further development of powerful optical tools, for which we are so largely indebted to Svedberg and his colleagues in Upsala (73), permit the observation of a series of moving boundaries, each revealing protein moving through the solvent with a characteristic electrophoretic mobility. In its most convenient form, the so-called Töpler schlieren (shadow) phenomenon (63, 74, 39) is employed to project on the photographic screen a pattern which can be resolved into a series of skewed probability curves, the area under each of which measures the concentration of the protein moving with each mobility. The mobility, u , calculated from the change with time of the center of mass of each area, is generally expressed in centimeters per second, when the protein is in an electric field with a gradient of 1 volt per centimeter. Such photographic diagrams for human, horse, bovine, and guinea pig serum are reproduced in figure 2, which thus illustrates differences between and similarities in the sera of different species.

Although there are differences in these schlieren diagrams, there are also very definite similarities, and the analysis of the proteins into electrophoretically different fractions is both convenient and valuable. At neutral reactions the fastest moving component of either plasma or serum is the albumin. Tiselius named the three other major components of serum the α -, β -, and γ -globulins. The last move most slowly in the electric field. Tiselius studied horse serum, but it was soon pointed out (72) that the behavior of human serum was comparable, and this is true in varying degrees of the other species that have been studied.

One of the great advantages of the electrophoretic method of analysis inheres in the convenience, reproducibility, and rapidity of the method. This may be illustrated by comparing the analysis of normal human serum into these four components that have been reported from three different laboratories, namely by Svensson (74) from Upsala, by Longsworth and his colleagues (44, 41, 40) from the Rockefeller Institute for Medical Research, and by Luetscher (42) from our laboratory. The ratio of the concentration of each globulin to the concentration of albumin in plasma is tabulated, thus avoiding both the factor of dilution and the factor of differences between plasma and serum. The good agreement between these results is quite gratifying and is none the less significant because each of the fractions revealed represents not a single protein but a population of proteins varying in size, shape, solubility, physiological and immunological function, and in many other respects.

The difference between the plasma and serum of the same species, depending on the absence in the latter of the characteristic fibrinogen peak, is represented in figure 3. Although fibrinogen is precipitated by lower concentrations of salt or of ethanol than the other serum proteins, it has

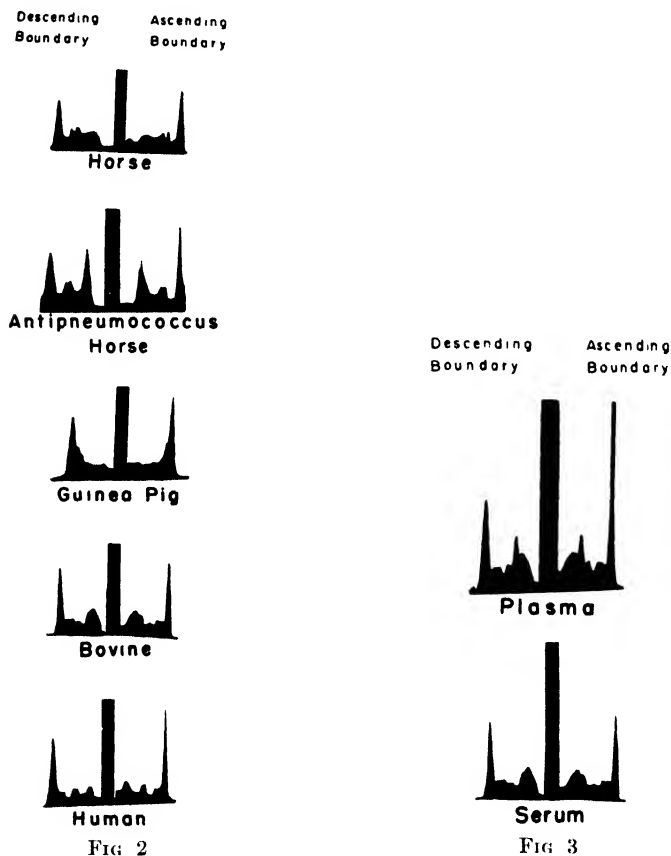


FIG 2 Electrophoretic schlieren patterns of human, bovine, guinea pig, and horse sera

FIG 3 Electrophoretic schlieren patterns of bovine plasma and serum

a higher electrophoretic mobility at neutral reactions than γ -globulin, presumably owing to its more acid isoelectric point.

A comparative electrophoretic study, such as that upon serum and plasma, may reveal the diminution in concentration of a protein, in this case fibrinogen, but cannot be used to prove its absence. A more satisfactory analysis of a separation than that depending upon differences in

solutions containing many protein components can be carried out with the separated precipitates. Thus the diagram of fibrinogen separated from bovine plasma by precipitation with ethanol at low temperature is reproduced in figure 4. Here we have but a single peak with a mobility close to that of fibrinogen in the original plasma. The absence of proteins moving with grossly different mobilities is demonstrated by such an electrophoretic analysis, but here, too, electrophoretic analysis alone cannot prove that but one protein is present, since even appreciable amounts of proteins with closely the same mobility as the main component could not readily be detected.

The electrophoretic diagram of γ -globulin prepared from bovine plasma is also reproduced in figure 4. Although completely different from fibrinogen, both in form and function, and conveniently characterized by its electrophoretic mobility as representing approximately 12 per cent of human and 18 per cent of bovine plasma, the γ -globulin fraction contains

TABLE I
Ratio of α -, β -, and γ -globulins to the albumin of human serum as revealed by electrophoretic analysis (42)

	α -GLOBULIN ALBUMIN	β -GLOBULIN ALBUMIN	γ -GLOBULIN ALBUMIN
Svensson	0.13	0.26	0.17
Longworth	0.12	0.23	0.20
Laetscher	0.11	0.21	0.19

a fair number of proteins differing in isoelectric point, in molecular weight, and in solubility.

Isoelectric precipitation

Just a century ago a French physiologist, Denis, reported to the group of protein chemists who were collaborating with Liebig in Giessen that certain of the proteins of the blood, which we now call globulins, or euglobulins, were soluble in salt solutions, but not in water (67). The precipitation of fractions of plasma proteins by dilution and acidification has often been employed since then. Globulins are, however, readily denatured in dilute solution, and so our practice in the separation and purification of globulins has generally been to avoid dilution, i.e., to avoid lower concentrations of salt and of protein than are absolutely necessary in order to effect the separation. It is accordingly an advantage that the very convenient methods of dialysis now available permit the precipitation of proteins, when sufficiently free of salt, near their respective isoelectric points.

The γ -globulin fraction of plasma is rich in euglobulin. Here again, however, there are differences between species. Thus, roughly one-third of the γ -globulin fraction of the horse, but over two-thirds of that of the cow, is euglobulin. The bovine γ -globulin, the electrophoretic analysis of which is reproduced in figure 4, despite its apparent uniformity, yielded proteins isoelectric near both pH 6 and pH 7. That there was euglobulin

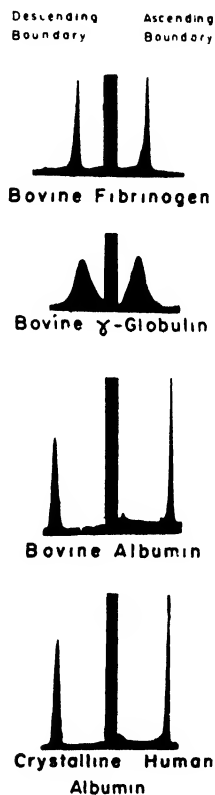


FIG 4 Electrophoretic schlieren patterns of human and of bovine plasma proteins purified by ethanol-water fractionation

isoelectric near pH 7 was first demonstrated in connection with the separation and purification of the pneumococcus antibody (13, 23). Euglobulins of isoelectric points near pH 5 and 6 have also previously been demonstrated (65, 21, 28). When the γ -globulin fraction is further fractionated by isoelectric precipitation into various euglobulins and pseudoglobulins, their electrophoretic mobilities prove not to be identical, although all are

generally within the area covered by the probability curve of the mean mobility of the γ -globulin

Sedimentation constants and molecular weights

Proteins with the same net charge should have the same electrophoretic mobility, provided they have the same size and shape. The size and shape of proteins which appear to be electrophoretically homogeneous are, however, often very different. The ultracentrifuge, for which we are indebted to Svedberg (73), is by far the most powerful tool that we have to permit discrimination between molecules of different sizes and shapes, but the ultracentrifuge alone does not permit evaluation of molecular weights. These can be determined from the rate of sedimentation in the ultracentrifuge, only provided the shape of the molecule or its diffusion constant is known. The sedimentation constants of the proteins are, however, revealed directly by ultracentrifugal analysis, and those recorded here are for solutions containing 1 per cent of protein in potassium chloride of ionic strength 0.2, corrected to the density and viscosity of water at 20°C. but not to zero protein concentration.

The largest part of the γ -globulin of horse serum sediments with a constant of 6.2×10^{-13} cm. per second in unit centrifugal field (1 cm. per sec.²), and bovine γ -globulin sediments with a constant estimated to be very nearly the same,—namely, 6.4×10^{-13} . Sedimentation constants of the order of 9, 12, 18, 32, and even 62×10^{-13} have been observed for euglobulins isoelectric at neutral reactions. This euglobulin fraction is rich in antipneumococcus antibody, and an increase in the amount of this and other antibodies is associated with a large increase in globulin, which often involves not only a relative increase but also an absolute increase in the total serum proteins. Certain of these antibodies have been considered γ -globulins, but some are thought to have slightly different mobilities (76, 77, 43, 60). Studies of Heidelberger and others (27, 34, 33) yielded a sedimentation constant for horse, pig, and cow antipneumococcus euglobulin of 18×10^{-13} , which, with measurements of free diffusion, suggested molecular weights of approximately 900,000, or close to six times the molecular weight of most other γ -globulins (27, 34, 33). The molecular weight of the γ -globulin of a sedimentation constant of 12×10^{-13} could, in fact, be smaller and that of 36×10^{-13} greater than 900,000.

Recently, Kabat (34) has observed that the molecules of this fraction, like fibrinogen, are very asymmetric in shape, revealing double refraction of flow, and it has been suggested that they seem to be polymers of smaller proteins conjugated end to end (79). These interesting results suggest how the process of immunization, resulting in molecules of large molecular weight and very small net charge at physiological reactions, can take place

TABLE 2

Estimated compositions, electrophoretic mobilities, and sedimentation constants of serum and plasma proteins

SOURCE OF PROTEINS	FIBRINO- GEN	GLOBULINS			ALBUMIN
		γ	β	α	
Proportion of total protein estimated by electrophoretic analysis, in per cent					
Human plasma (42)	6	12	13	7	62
Bovine plasma	18	18	8	16	40
Human serum		13	14	7	66
Bovine serum		22	10	20	48
Horse serum		24	21	13	42
Electrophoretic mobilities of 1 per cent protein in phosphate buffer solutions of 0.2 ionic strength and pH 7.7 at 0°C.: $u_{20}^{1\%} \times 10^5$					
Human plasma (42)	1.9	0.9	2.7	3.8	5.1
Bovine plasma fractions (8)	2.1	1.6			5.2
Horse serum fractions (9)*.		1.9	2.9	3.9	$\begin{cases} 4.5 \\ 5.3 \end{cases}$
Sedimentation constants† of 1 per cent protein in potassium chloride solutions of 0.2 ionic strength: $s_{20,w}^{1\%} \times 10^{13}$					
Human plasma fractions..					3.9
Bovine plasma fractions (8)	7.6	$\begin{cases} 6.4 \\ 17 \end{cases}$			4.0
Horse serum fractions (27, 34, 33, 9)		$\begin{cases} 6.2 \\ 17 \end{cases}$	6.3	$\begin{cases} 6.8 \\ 17 \end{cases}$	3.9

* The values in serum are somewhat different. Studies thus far completed by J. M. Newell and S. H. Armstrong, Jr., suggest 1.7 for γ , 3.4 for β , 4.1 for α , and 5.6×10^{-5} for the total albumin. The crystalline carbohydrate-free albumin has a mobility of 5.3 and the 5.5 per cent carbohydrate-containing crystalline albumin of 4.5×10^{-5} cm.² per volt-second.

† Svedberg and Pedersen (73) give a value of $s_{20,w}$ reduced to zero protein concentration of 7.1×10^{-13} for both total horse serum globulin obtained by half-saturation with ammonium sulfate and for electrophoretically prepared γ -globulin from normal human serum. They give the value 4.46×10^{-13} for the sedimentation constant of horse serum albumin, obtained by averaging the value for the A and B fractions prepared by Kekwick (35). McFarlane's (45) studies on diluted normal serum give 6.5-6.7 for horse and cow and 5.7 for human total globulin, and 4.2-4.4 for horse and cow and 3.9×10^{-13} for human albumin. The last value is in exact agreement with our study of purified human albumin.

without serious disturbances of water balance. These large globulins, existing close to their isoelectric points, would presumably make the smallest contributions to osmotic pressure of any of the plasma proteins.

Thus far certain antibodies have been located in the γ -globulin fraction. Another concern of the immunologist is complement, essential in certain antigen-antibody reactions such as the hemolysis of red blood cells by specific antisera. Long since recognized as associated with the physical-chemical state of the serum proteins, it would appear to depend upon the presence of but small amounts of a few components, one of which has recently been obtained as a euglobulin. Separated from serum by concentrations of ammonium sulfate generally employed in precipitating the γ -globulin fraction, and having a sedimentation constant of 6.4×10^{-13} , characteristic of most globulins, it has an electrophoretic mobility in 0.2 *M* phosphate buffer at pH 7.7 of 2.9×10^{-5} , possibly more characteristic of a β - than a γ -globulin. Its isoelectric point is close to pH 5.2 and it has been separated from other components of complement because it is quite insoluble in phosphate buffers of pH 5.2 and ionic strength 0.02, but soluble at this reaction at slightly higher ionic strengths.⁵

Prothrombin is generally precipitated from plasma with the γ -globulins, and its isoelectric point is also in the neighborhood of pH 5, at which point it is frequently separated by dilution of plasma and acidification. Its further purification from fibrinogen and other euglobulins has been carried out by the addition of calcium bicarbonate (50), or by isoelectric precipitation (10, 11, 68, 21). It is not our purpose in this survey to discuss the best methods for the preparation of each plasma protein, but rather to locate as on a contour map the various proteins which have thus far been identified, because of either their physical characteristics or their physiological functions.

As methods of separating the very labile protein molecules of physiological significance improve, it is probable that the well-characterized components of plasma will increase. Knowledge of the physical and chemical characteristics of such molecules should in turn increase our insight into the nature of their functions and interactions.

The γ -globulin fraction contains molecules which have not thus far been separated as euglobulins by exhaustive dialysis in the neighborhood of their isoelectric points and which may therefore be called pseudoglobulins. The γ -pseudoglobulin of horse serum has been very carefully studied and found to have an isoelectric point of 6.3, an electrophoretic mobility at 0°C. in phosphate buffer of 0.2 ionic strength at pH 7.7 of 1.9×10^{-5} , a sedi-

⁵ The so-called "mid piece" and "end piece" of guinea pig serum have recently been purified and characterized by L. Pillemer of Western Reserve University, working in this laboratory.

mentation constant, $s_{20,w}$, of 6.2×10^{-13} , a diffusion constant, $D_{20,w}$, of 4.1×10^{-7} , and a partial specific volume of 0.730. Its molecular weight is thus calculated to be 142,000 (9). This fraction of γ -globulin, though it has a very small net charge, has proved to have the greatest influence in increasing the dielectric constant of solutions of any of the plasma proteins thus far investigated (14, 7, 58). Its electrical asymmetry is thus very great and this should result in strong interactions with ions and with other proteins.

IV. THE β -GLOBULIN COMPONENTS OF PLASMA

Globulins of electrophoretic mobility greater than γ -globulin and fibrinogen have been termed α - and β -globulins by Tiselius. In plasma or serum, β -globulins reveal a double peak (figure 2), suggesting the presence of two or more groups of molecules. Upon separation of the serum and reprecipitation, this double peak often appears to vanish. Purified globulins have, however, been prepared with mobilities intermediate between those characteristic of the β - and γ -globulins.

Whereas γ -globulin preparations are often nearly colorless, β -globulin fractions are often highly colored, some blue (21), some deep yellow (75), some rich in lipoids, and some rich in other organic molecules associated with this group of proteins. Their concentration in sera increases in lipid nephrosis, cirrhosis of the liver, and other pathological conditions (24, 72, 2, 41, 42, 44, 40).

β -Globulins are precipitable by neutral salts (9) or by alcohols (8) at concentrations greater than those necessary for the precipitation of γ -globulins. Their electrophoretic mobilities at neutral reaction reflect greater net charge and more acid isoelectric points. In terms of equation 1 it does not follow that the "salting out" constant, K_s , is smaller for β - than for γ -globulins, although this may be the case. More probably, the solubility coefficient, β , is greater. Separation of globulins of more acid isoelectric points can thus be accomplished either by increasing the concentration of the precipitant or by bringing the acidity closer to the isoelectric point of each protein.

These generalizations apply equally to albumins, pseudoglobulins, and euglobulins. Euglobulins are, as we have seen, precipitable near their isoelectric point in the absence of salt. Such separations are, however, often carried out more satisfactorily after preliminary fractionation, so as to reduce interactions between proteins of very different isoelectric point. Evidence that there are euglobulins in serum differing in isoelectric points has come not only from electrophoretic but also from solubility (13, 65, 21, 23) and immunological (18, 34, 36, 27, 33, 43, 60) studies. Most proteins isoelectric at neutral reactions are, as we have seen, part of the

γ -globulin fraction. On the other hand, the one component of complement (see footnote 5), prothrombin, and the very characteristic blue-green, jelly-like euglobulin, with an isoelectric point close to pH 5 (described by Green as P_1), appear to move in the electric field "with the β -globulin," as does a globulin component with an isoelectric point near pH 6 (42).

Those proteins characterized in the electrophoretic analysis of whole serum or plasma as β - or γ -globulins would thus appear to consist of various euglobulins and pseudoglobulins, of slightly different isoelectric point and acid- and base-combining capacities. Some of these are presumably present in but small amount, and as in the case of the γ -globulin, some may have molecular weights that are both higher and lower than is characteristic of most of the β -globulin. A fair amount of the β -globulin of horse serum, upon purification, appears to have a lower sedimentation constant in the presence of high protein concentration, presumably owing to dissociation of β -globulin into components of smaller molecular weights (45, 73, 9). The sedimentation constants and molecular weights of most of the globulin fractions would appear, however, to be closely similar (55, 45, 35), but this may not be true of their shapes (12, 56), phosphorus or carbohydrate contents, dielectric dispersion curves, or other chemical or physico-chemical properties which reflect in more detail the fine structure of molecules upon which their physiological functions presumably depend.

V. THE α -GLOBULIN COMPONENTS OF PLASMA

The chemical separation of α - and β -globulins from γ -globulins is far simpler than the separation of α - and β -globulins from each other. α -Globulin is generally, although not always, represented by a single peak, apparently moving with uniform electrophoretic mobility in serum or plasma. Precipitable by higher concentrations of ammonium sulfate than β - or γ -globulin (9), α -globulin consists, in the main, of water-soluble pseudoglobulins, isoelectric near pH 5. There are euglobulins, however, which move with the electrophoretic mobility of the α -globulin fraction; among them is another component of complement, the so-called "end piece", which has been separated in a state of considerable purity, has an electrophoretic mobility of 4.2×10^{-5} , and contains 10.3 per cent of carbohydrate.⁵ The experimental conditions which it was necessary to achieve in order that this component, previously believed to be pseudoglobulin in nature, would separate as a euglobulin suggest that other substances associated with the α -globulin may also ultimately be isolated as euglobulins. It is possible that the so-called mucoglobulin (28), as well as certain other carbohydrate-rich globulins (71, 35), and perhaps also the crystals reported by Jameson (30, 31), may have been α -globulins.

The α - and β -globulin fractions of plasma are often more labile than

either the γ -globulin or the albumin fractions. They also contain a large proportion of the chromogenic groups associated with serum proteins. It is not impossible that their lability is related to changes in these associated groups.

α -Globulin preparations, uniform electrophoretically, have revealed diverse sedimentation constants suggesting components of different molecular weights (43, 60, 9). In how far these components represented β -globulin or albumin rich in carbohydrate impurities remains uncertain, but in the case of both fractionated horse and bovine serum proteins, ultracentrifugal analyses suggested far higher concentrations of molecules of the size of the albumins than electrophoretic analyses indicated were present.

The functions of the α - and β -globulins are presumably different from those of either the γ -globulins or the albumins. They are different immunologically and most antibodies have been associated with the γ fraction, which increases in amount with immunity. In febrile conditions, on the other hand, it is the α -globulin fraction which largely increases (44, 41, 40). In lipid nephrosis, in which fibrinogen increases in the plasma, so also do the α - and β -globulins, whereas the γ -globulin and albumins are greatly diminished. Conversely, albumin, and to some extent γ -globulin, are found in lymph (57) and perhaps in other body fluids (42) in larger amounts than α - and β -globulins, which would seem to leave the blood less readily (44, 41, 40). More exact characterization of these various plasma proteins, under varying physicochemical conditions, and their availability as purified proteins in adequate amounts for physiological and clinical investigation should greatly increase our knowledge, not only of the nature and the function of blood proteins, but also of kidney and tissue permeability.

VI. THE ALBUMIN COMPONENTS OF PLASMA

Albumins are by definition soluble in water. There are, however, many water-soluble proteins in serum and plasma which are far more closely related to the globulins than to the albumins and which we have termed pseudoglobulins. They move in the electric field with the mobility characteristic of globulins and have sedimentation constants characteristic of globulins but are often completely "salted out" only at concentrations which effect the precipitation of albumins.

Serum albumins are precipitated by neutral salts or by organic solvents at higher concentrations than are most globulins. One of the conventional methods for their preparation is to half-saturate plasma with ammonium sulfate, and to purify the albumin from the filtrate. Whereas very little albumin is precipitated in neutral solution by half-saturation with ammonium sulfate, that is to say, from a 2 molal solution of ammonium

sulfate at ordinary temperatures, the filtrate always contains globulin, some rich in carbohydrate (28, 76, 66) and some closely related to the α - and β -globulins.

The further purification of the serum albumins is accomplished either by increasing the concentration of the precipitant or by increasing the acidity. Serum albumin is isoelectric near pH 4.8. This is true at least for the albumin of human, horse, and bovine serum or plasma. The albumins in the blood are thus far from their isoelectric point, are combined with more base than are the globulins, and therefore would have a greater electrophoretic mobility even were they of the same molecular weight as the globulins.

The molecular weight of most albumins is in the neighborhood of 70,000, or approximately half of that of most of the globulins, and this also would lead to greater electrophoretic mobilities, as well as to greater osmotic pressures per gram of protein. Although albumins have a greater net charge at neutral reactions, as well as a greater number of charged groups in the isoelectric condition, these are arranged with far greater symmetry. As a result, albumins have the smallest electric moments thus far observed for any proteins. They should thus interact less with other proteins, as well as with salts. Albumin solutions are also more limpid and exhibit the Tyndall effect to but a negligible extent. They are, moreover, far less readily denatured than are most other proteins.⁶

Immunologically, also, the albumins may be contrasted with the globulins. The purified albumins may be sharply differentiated from the γ -globulins, the chief antibodies of the blood stream, by serological methods (32, 28).

Although albumins can thus be contrasted with globulins, the albumins of different species and indeed the albumins of the same species are not homogeneous. This may be shown chemically or immunologically. Thus the purified albumins of man, horse, and cow seem to be immunologically distinct from one another (32). The albumins of the horse crystallize readily from serum, while those of man crystallize only after being separated from α - and β -globulins and lipoids. None the less, the electrophoretic mobilities, sedimentation constants, and many other properties of the albumins of different species are closely comparable (table 2).

Different albumins may be demonstrated to be present in any of these species. The readily crystallizable horse serum albumins have been the most studied (69, 70, 7, 14, 28, 35, 46, 58, 66). Albumin may be crystallized

⁶ Indeed, it is possible to purify albumins of certain other protein impurities by permitting the latter to denature in 15 or 20 per cent ethanol at pH 4.8 at temperatures slightly above zero, separating the precipitate, and, in the case of human protein fractions, crystallizing the albumin.

if it is carbohydrate-free or if it contains as much as 5.5 per cent of carbohydrate (46). Among the crystalbumins, a further separation can be made, in that a portion thereof may be crystallized from salt-free solution as a sulfate⁷ (46), although the crystal forms of the insoluble and soluble sulfates appear to be identical. Another type of crystalline albumin, hemocuprein, containing copper, has been isolated by Mann and Keilin (47) and confirmed in our laboratory. Its function remains to be discovered; it is not respiratory and may well be enzymatic. On the other hand, the choline esterase and phosphatase of serum, the lipase and iodine-containing proteins, as well as other hormones and enzymes which separate with the albumin fraction, remain to be crystallized.

The electrophoretic mobilities of all of the serum albumins are not identical. That of hemocuprein has not been reported, although its molecular weight is said to be smaller than that generally ascribed to serum albumin. Whereas the sedimentation constant of the crystalline albumin containing 5.5 per cent of carbohydrate appears to be very nearly that of carbohydrate-free albumin, its electrophoretic mobility has been demonstrated to be appreciably smaller at pH 7.7, that is to say, at the reaction at which all of the electrophoretic mobilities thus far reported in this communication were determined.

It is probable that other crystalline serum albumins will in time be isolated and their properties and functions accurately determined. It has long been known that even the beautifully crystalline serum albumin of the horse does not consist of a single chemical individual as judged by solubility (69, 70) or by dielectric studies (14, 7, 58), even though homogeneous as to molecular weight or electrophoretic mobility at neutral reactions.

The crystallization of salt-free albumin sulfate was carried out near pH 4 (46).⁷ At this reaction not only horse but also bovine and human albumin revealed components of different electrophoretic mobility, the faster moving albumin constituting nearly two-thirds of the total (42). In certain pathological conditions in man (among them lipoid nephrosis and cirrhosis of the liver), however, electrophoretic analysis of both serum and urinary albumins showed a greater loss of the faster moving albumin from the blood, leaving the slower component preponderant. Studies of the dielectric properties of the albumin separated from the urine of these conditions yielded a fraction with a dielectric increment far greater than that generally ascribed to serum albumin (17). Since the ratio of albumin components appeared to be the same in blood and in urine, differential per-

⁷ An observation reported by J. D. Ferry at the Division of Biological Chemistry at the Ninety-sixth Meeting of the American Chemical Society, held at Milwaukee, September 7, 1938.

meability for excretion was not assumed, but rather a differential production of the two albumins in the body (42).

Clearly, further studies are necessary and possible not only on the loss under pathological conditions from the blood to the urine or the tissues of various proteins, but of their various functions in the blood. The albumins and γ -globulins would appear to be lost more readily than the α - or β -globulins. On the other hand, the influence of the albumins on water balance must be greater than that of the other proteins. Whereas the albumins represent over 60 per cent of the proteins of normal human plasma, they give rise to an even greater proportion of the blood osmotic pressure by virtue of their smaller size and larger net charge. These properties and their lower viscosity, more symmetrical shape, and charge distribution, and their far greater solubility and stability in solution separate them in property and function from the more labile, asymmetric, and viscous globulin fractions.

The preparation of large amounts of relatively pure albumin is now possible. Although knowledge of the conditions for the crystallization of various serum albumins, especially those of horse serum (28, 35, 46; footnote 7), from concentrated solutions of sulfates or phosphates has been greatly extended in recent years, this method is not susceptible to large-scale preparation. The method of fractionating serum or plasma proteins by equilibration across membranes with ethanol-water mixtures of controlled pH, ionic strength, and temperature, which also yields fibrinogen and γ -globulin fractions approaching homogeneity with respect to both size and net charge, is especially advantageous in the separation and purification of the albumins (8). Schlicren diagrams of these fractions separated from plasma are illustrated in figure 4. Whereas fibrinogen and all the γ -globulins, as well as a fair amount of the α - and β -globulins, are largely precipitated by 40 per cent ethanol at -5°C ., the solubility of these is further reduced if the acidity is increased to approximately pH 5.5, preferably by acetate or carbonate buffers.⁸ The albumin remaining in solution in 40 per cent ethanol at pH 5.5 at -5°C . is largely precipitated from this solvent at pH 4.4-4.8, as is also the small amount remaining in solution at this temperature by concentration at -15°C . Albumin, both human and bovine, has been prepared by this method and is pure both electrophoretically and in the ultracentrifuge, and there is practically no

⁸ Equilibration with carbonate buffers, which we are further developing in collaboration with Dr. R. M. Ferry, has the advantage that, at constant carbon dioxide pressure, increase in acidity is produced by diminution of free base. Its removal from the protein solution by dialysis through membranes is favored by the Donnan equilibrium, whereas the introduction of other acids to the protein phase by diffusion across membranes is impeded by the Donnan equilibrium.

upper limit to the amounts of this material (as of fibrinogen and the γ -globulins) that can readily be made available.

The albumin precipitable from 40 per cent ethanol is readily dried and is a colorless white powder, free of reducing substances and indeed of other organic and inorganic molecules for which we have thus far tested. It readily dissolves in water, yielding clear solutions even at concentrations greater than 25 per cent. In our experience no precipitate appears in such solutions, even after they have stood for protracted periods of time. Indeed, albumin purified in this way appears to be stable for short periods of time even in 20 per cent ethanol at room temperature.⁶

Albumin that appears to be uniform both electrophoretically and ultracentrifugally can be further fractionated by ethanol-water mixtures. Thus, a fraction of the albumin which contains traces of globulin has a high heat of solution and dissolves as an oil, with increase in temperature, and is insoluble in 25 per cent ethanol at pH 4.8 and 0°C.; another fraction is soluble even in 20 per cent ethanol at -5°C., but insoluble in 40 per cent ethanol at -5° and -15°C. The fractionation of albumins in alcohol-water mixtures by change in temperature is the more interesting because the heat of solution in these solvents is opposite in sign⁹ to that in the concentrated ammonium sulfate solutions in which albumins have heretofore generally been fractionated and crystallized. Human albumin is readily crystallized from ammonium sulfate after such fractionation.

The availability of bovine albumins is making possible a systematic study of the chemical modification of their free groups by reagents which are yielding proteins of new physical-chemical properties and new immunological behavior. Experiments which will be reported elsewhere are now under way in which amino groups are replaced by guanidine,¹⁰ acetyl, urcide, or other groups and in which the other basic groups or the phenolic hydroxyl groups of the proteins are modified. Modification of carboxyl groups, although often leading to changed antigenicity, has seemed a less promising approach to present needs, since the preparation of proteins of modified antigenicity but of unmodified, or increased, rather than decreased, osmotic pressure at neutral reactions is our aim. Such modified

⁹ The theoretical consideration of these differences, involving the theory of "salting out" and of electrostatic interaction, will be considered elsewhere.

¹⁰ Treatment of the albumin of horse or bovine serum with methylisourea (for a considerable supply of which we are indebted to the American Cyanamid Company) has yielded euglobulins. Thus, W. L. Hughes and the author have succeeded in separating water-insoluble, salt-soluble globulin from albumin, the amino groups of which had been guanidinated. The immune properties had, however, not been greatly changed by this complete change in solubility behavior, nor probably had the net charge or electric moments of the molecules.

proteins derived from bovine plasma might prove especially suitable for transfusion after a period of careful clinical investigation.

Human albumin, as well as bovine albumin, can be prepared by the above procedures even from plasma or serum containing so much hemolyzed blood as to render it unsuitable for transfusion. Such purified albumin in solution, freed from the more labile and more readily denatured fibrinogen and globulins, should prove useful for a number of therapeutic purposes, particularly in the treatment of shock, due to acute loss of blood. There is good reason to believe that its marked osmotic effect will draw water into the blood vessels, raise the total blood volume, and thus relieve the condition, provided it is not too rapidly lost from the blood stream because of its smaller molecular weight. From a physicochemical point of view, human and bovine albumin are remarkably similar molecules and the latter could be used as a substitute for human albumin provided no clinical contradictions are demonstrated.

The multiplicity of functions performed by the plasma proteins remains larger than the number of pure proteins that have thus far been isolated from serum or plasma. The methods would appear to be available, however, for the purification of any protein for which there are adequate methods of bioassay. The procedures now being carried out on a large scale, with both the human and the bovine plasma, yield, in separate fractions, fibrinogen, three γ -globulins, prothrombin, α - and β -globulins, and the serum albumins. These developments, which permit the separation of molecules of closely similar properties, depend less on the discovery of new chemical reagents than upon the development of the theory and the more precise control of the conditions upon which purifications depend and on increasing knowledge of the physicochemical properties of the proteins.

SUMMARY

1. The diverse nature of the plasma proteins is discussed and the physicochemical bases for their characterization defined.
2. The physiological functions of certain of the proteins are considered in relation to their chemical functions.
3. The large-scale preparation of the different proteins of human and of animal plasma in purified states renders possible not only chemical but also clinical investigations of their functions and possible therapeutic uses.
4. Although albumins may be lost from the circulation more readily than certain globulins, their smaller size and greater net charge lead to greater osmotic effects. The high osmotic pressure, low viscosity, and great stability of albumin solutions would appear to render them the most useful of the plasma proteins for the treatment of some, but not necessarily all, conditions associated with diminished plasma volume.

5. Comparative studies on the physical, chemical, and immunological properties of bovine, of chemically modified bovine, and of human albumins and globulins are considered.

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SOME ASPECTS OF THE THERMODYNAMICS OF STRONG ELECTROLYTES FROM ELECTROMOTIVE FORCE AND VAPOR PRESSURE MEASUREMENTS

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Received December 7, 1940

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For a number of years the authors have been engaged separately in investigations of the thermodynamic properties of strong electrolytes by the electromotive force and vapor pressure methods. The results, which have appeared in numerous papers in different journals, have not yet been systematically coordinated, and a critical survey of this work is now desirable.

¹ Sterling Fellow, Yale University, 1939-40.

An adequate consideration of all the many excellent contributions to this subject would have extended the review to a lengthy treatise; we have therefore limited the field to a number of topics in which we have been particularly interested. To this end we shall focus attention on the partial molal free energy of electrolytes, or its derived function, the activity coefficient, and the variation of the free energy with changes in temperature, pressure, electrolyte concentration, and medium composition. Early in the review, a detailed consideration of these variations will be made in the case of hydrochloric acid, an electrolyte about which sufficient is known to exemplify each of the four variations.

Numerous data will be given for the activity coefficients of electrolytes in water at 25°C., and their significance will be considered in relation to different theoretical treatments of electrolytic solutions. Finally, attention will be directed to the calculation of activity coefficients over a temperature range.

We feel confident that this summary of the subject will be of value in a number of ways. The tables of data (some of which have not been published previously) will be of considerable practical value to those engaged in experimental work. We hope that this material will also be of use in testing proposed theories of concentrated solutions. Furthermore, at intervals in the course of the review, we shall draw attention to problems which invite further experimental or theoretical investigation.

I. FUNDAMENTAL EQUATIONS

A comprehensive treatment of this subject requires a knowledge of the variation of some fundamental quantity, such as the partial molal free energy, \bar{F} , or the activity coefficient of an electrolyte as a function of the four important variables: temperature, the concentration of the electrolyte, the composition of the solvent, and pressure.

The fundamental relationships between the partial molal free energy and the activity coefficients on the N -, m -, and c -scales may be stated as follows:

$$\bar{F} = \bar{F}_N^0 + \nu RT \ln f_{\pm} N_{\pm} = \bar{F}_m^0 + \nu RT \ln \gamma_{\pm} m_{\pm} = \bar{F}_c^0 + \nu RT \ln y_{\pm} c_{\pm} \quad (1)$$

where \bar{F}_N^0 , \bar{F}_m^0 , and \bar{F}_c^0 are the standard free energies, f_{\pm} , γ_{\pm} , and y_{\pm} are the mean activity coefficients on the mol fraction, molality, and molarity scales, respectively, and ν is the number of ions formed by the dissociation of one molecule of electrolyte.²

² If an electrolyte, $C_{\nu+}A_{\nu-}$, dissociates into ν ions, of which ν_+ are cations and ν_- are anions, and n_2 mols of electrolyte are dissolved in n_1 mols of solvent, the cation,

Upon introducing the usual convention that $f_{\pm} = \gamma_{\pm} = y_{\pm} = 1$ at infinite dilution of the electrolyte, the equations

$$\bar{F}_N^0 = \bar{F}_m^0 + \nu RT \ln 1000/M_1 = \bar{F}_c^0 + \nu RT \ln 1000d_0/M_1 \quad (2)$$

and

$$\ln f_{\pm} = \ln \gamma_{\pm} + \ln (1 + \nu m M_1/1000) \quad (3)$$

$$\ln f_{\pm} = \ln y_{\pm} + \ln (d/d_0 + c(\nu M_1 - M_2)/1000d_0) \quad (4)$$

$$\ln \gamma_{\pm} = \ln y_{\pm} + \ln (d/d_0 - cM_2/1000d_0) \quad (5)$$

relating these functions may be obtained. In these equations M_1 and M_2 are the molecular weights of solvent and solute, respectively, and d_0 and d are the densities of solvent and solution.

The \pm subscripts to f , γ , and y are inserted because the quantities are mean activity coefficients, related to the individual ionic activity coefficients by an equation of the form of 1a. As we shall not discuss individual ionic activity coefficients, it will now be possible, in the interests of simplicity, to omit the subscripts from f , γ , and y . This simplification cannot be made for m_{\pm} , and it is important to note that only in the case of a 1-1 electrolyte does m_{\pm} equal m .

Equations 1 and 3 will be used frequently in this review, the former to express experimental results on the molality scale and the latter in comparisons of experimental results with theoretical equations based on the N -scale. Thus:

$$\bar{F} - \bar{F}^0 = \nu RT \ln \gamma m_{\pm} = \nu RT \ln \gamma m + RT \ln (\nu_+^+ \nu_-^-) \quad (6)$$

N_+ , the anion, N_- , and the mean ionic mol fractions, N_{\pm} , are defined by the equations:

$$N_+ = \nu_+ N$$

$$N_- = \nu_- N$$

and

$$N_{\pm} = (N_+^{\nu_+} N_-^{\nu_-})^{1/\nu} = (\nu_+^{\nu_+} \nu_-^{\nu_-})^{1/\nu} N \quad (1a)$$

where

$$N = \frac{n_2}{n_1 + \nu n_2}$$

On the molality (mols of solute per 1000 g. of solvent) and molarity (mols of solute per 1000 cc. of solution) scales, we have similar definitions for the mean ionic molality, m_{\pm} , and the mean ionic molarity, c_{\pm} , namely:

$$m_{\pm} = m(\nu_+^{\nu_+} \nu_-^{\nu_-})^{1/\nu} \quad \text{and} \quad c_{\pm} = c(\nu_+^{\nu_+} \nu_-^{\nu_-})^{1/\nu}$$

and for aqueous solutions:

$$\ln \gamma = \ln f - \ln (1 + 0.018vm) \quad (7)$$

The temperature coefficients of $(\bar{F} - \bar{F}^0)$ and $\ln \gamma$ are related to the partial molal heat content relative to infinite dilution, $\bar{H}_2 - \bar{H}_2^0 \equiv \bar{L}_2$, of the electrolyte by the well-known thermodynamic equations:

$$\left. \frac{\partial(\bar{F} - \bar{F}^0)/T}{\partial T} \right]_{P,m} = -\frac{(\bar{H}_2 - \bar{H}_2^0)}{T^2} = -\frac{\bar{L}_2}{T^2} \quad (8)$$

and

$$\left. \frac{\partial \ln \gamma}{\partial T} \right]_{P,m} = -\frac{\bar{L}_2}{\nu RT^2} \quad (9)$$

The pressure coefficients are given by:

$$\left. \frac{\partial(\bar{F} - \bar{F}^0)}{\partial P} \right]_{T,m} = \bar{V}_2 - \bar{V}_2^0 \quad (10)$$

and

$$\left. \frac{\partial \ln \gamma}{\partial P} \right]_{T,m} = \frac{\bar{V}_2 - \bar{V}_2^0}{\nu RT} \quad (11)$$

where $(\bar{V}_2 - \bar{V}_2^0)$ is the relative partial molal volume of the solute. Further, we shall require the equation:

$$\left. \frac{\partial \bar{L}_2}{\partial T} \right]_{P,m} = \bar{J}_2 = (\bar{C}_{p_2} - \bar{C}_{p_2}^0) \quad (12)$$

where \bar{J}_2 is the partial molal heat capacity of the solute relative to infinite dilution. In these equations the subscript 2 has been introduced to denote that these partial molal quantities refer to the electrolyte, the subscript 1 being reserved for quantities referring to the solvent.

When the free energy of the solvent is measured directly, as in freezing-point determinations, it is convenient to define two osmotic coefficients. The rational osmotic coefficient, g , on the N -scale, is defined by:

$$\bar{F}_1 - \bar{F}_1^0 = gRT \ln N_1 \quad (13)$$

where N_1 is the mol fraction of solvent. The practical osmotic coefficient, ϕ , on the m -scale, is, for a single salt in aqueous solution,

$$\bar{F}_1 - \bar{F}_1^0 = \phi RT \frac{\nu m}{55.51} \quad (14)$$

where m is the molality of solute. In terms of the activity of the water and the vapor pressure:

$$\phi = -\frac{55.51}{vm} \ln a_1 = -\frac{55.51}{vm} \ln \frac{p}{p_0} \quad (15)$$

p being the vapor pressure of the solution and p_0 that of the solvent.

The practical osmotic coefficient may be derived from the practical activity coefficient by means of the relation:

$$\phi = 1 + 1/m \cdot \int m d \ln \gamma \quad (16)$$

and the reverse operation may be performed by means of the relation:

$$\begin{aligned} -\ln \gamma &= h + \int h d \ln m \\ &= h + 2 \int h / \sqrt{m} \cdot d\sqrt{m} \end{aligned} \quad (17)$$

where $h = (1 - \phi)$.

Besides these fundamental thermodynamic relations, we shall require the important equations which result from the Debye and Hückel (17) interionic attraction theory. The limiting equations of this theory for f , \bar{L}_2 , and J_2 may be expressed by the following simple equations:

$$\log f = -S_{(f)} \sqrt{\Gamma} \quad (18)$$

$$\bar{L}_2 = S_{(\bar{L}_2)} \sqrt{\Gamma} \quad (19)$$

$$J_2 = S_{(J_2)} \sqrt{\Gamma} \quad (20)$$

where $S_{(f)}$, $S_{(\bar{L}_2)}$, and $S_{(J_2)}$ are the theoretical limiting slopes, and $\Gamma (= \Sigma c_i z_i^2)$ is the ionic concentration of electrolyte.³ Values of the slopes for aqueous solutions at different temperatures are given in table 1.

We shall also require the theoretical equation for the variation of f with electrolyte concentration which includes the effect of the restriction of attraction between the ions due to their finite size. Thus,

$$\log f = -\frac{S_{(f)} \sqrt{\Gamma}}{1 + A \sqrt{\Gamma}} \quad (21)$$

If A is determined, the mean distance of approach of the ions in Ångström units, \bar{a} , may be obtained from the data in the last column of table 1, in

³ It has been customary to call Γ the ionic concentration of electrolyte, but by analogy with the terms *molar* and *molal*, $\Sigma c_i z_i^2$ and $\Sigma m_i z_i^2$ would be denoted as the *ionic* and *ional* concentrations, respectively.

which the theoretical values of A/d for aqueous solutions are recorded over a considerable temperature range. The values of the constants employed in these computations are given at the bottom of the table. The values of the dielectric constant necessary for the calculation were computed from Wyman's (140, 143) numerical equation, also given at the foot of table 1.

TABLE 1

Limiting slopes of the Debye and Huckel theory for single electrolytes in water.

Values of A/d . The magnitude of the product of the valences of the ions of the electrolyte = $|z_1 z_2|$. $\nu = \nu_1 + \nu_2$

t °C	$S(f)$ $ z_1 z_2 $	$S(\bar{L}_\nu)$ $\nu z_1 z_2 $	$S(\bar{J}_\nu)$ $\nu z_1 z_2 $	A/d
0	0 3446	153	3 40	0.2294
5	0 3466	160	3 67	0 2299
10	0 3492	190	3 96	0 2305
15	0 3519	210	4 22	0 2311
20	0 3549	231	4 48	0 2318
25	0 3582	254	4 72	0 2325
30	0 3616	278	4 96	0 2332
35	0 3653	303	5 19	0 2340
40	0 3692	329	5 44	0 2348
50	0 3777	385	5 89	0 2366
60	0 3871	446	6 30	0.2386
70	0 3973			0 2407
80	0 4083			0.2429
90	0 4200			0 2452
100	0 4325			0.2476

Constants: N = Avogadro's number = 6.061×10^{23} .

e = electronic charge = 4.774×10^{-10} e.s.u.

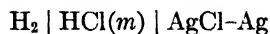
k = gas constant per molecule = 1.372×10^{-16} ergs deg.⁻¹

$T = t + 273.1$.

$D = 78.54[1 - 0.00460(t - 25) + 0.0000088(t - 25)^2]$.

II. HYDROCHLORIC ACID

Owing to the ease and accuracy with which the electromotive force of the cell



can be measured in water and other solvents, it has been possible to investigate the thermodynamic properties of hydrochloric acid extensively. With the aid of these results, a comprehensive view of the properties of a single electrolyte, as a function of the temperature, the pressure, the com-

position of the solvent, and the concentration of the electrolyte, may be obtained without introducing difficulties due to the presence of ions of charge greater than unity.

From the fundamental equation of this cell,

$$E = E^0 - 2.3026RT/F \cdot \log \gamma m \quad (22)$$

the activity coefficient of the acid, γ , at a molal concentration, m , may be computed if the standard potential, E^0 , is known. The methods of evaluation of E^0 from the electromotive forces, although very important, have been described in great detail elsewhere (32, 34, 35, 51, 59) and need not

TABLE 2

Standard potentials of the cell: $H_2(1 \text{ atm.}) | HCl(m) | AgCl-Ag$
 Values of $2.3026RT/F$: $R = 1.9869 \text{ calories } (15^\circ)$; $T \text{ (ice point)} = 273.1^\circ$;
 $F = 96,500 \text{ coulombs}$

t	$2.3026RT/F$	E^0
$^\circ\text{C.}$		
0	0.05419	0.23634
5	0.05519	0.23392
10	0.05618	0.23126
15	0.05717	0.22847
20	0.05816	0.22551
25	0.05915	0.22239
30	0.06015	0.21912
35	0.06114	0.21563
40	0.06213	0.21200
45	0.06312	0.20821
50	0.06412	0.20437
55	0.06511	0.20035
60	0.06610	0.19620

be considered here. We shall restrict this treatment to the presentation of the results, rather than to the technical methods by which they were derived.

Table 2 contains the values of E^0 in aqueous solutions from 0° to 60°C. , obtained by Harned and Ehlers (44). The values of $2.3026RT/F$ used by them and computed from the universal constants given in the *International Critical Tables* (63) are also given in the table. It is of some importance to note that the values of R , T (ice point), and F , compiled by Birge (11), would lead to somewhat different values of E^0 (e.g., 0.22223 instead of 0.22239 volt at 25°C.) (60). This will also affect the values of γ and of other derived quantities to a lesser extent.

The mean activity coefficient of the acid in water, from the results of

TABLE 3
The activity coefficient of hydrochloric acid in water at various temperatures† (44)

m	ACTIVITY COEFFICIENT													
	0°	5°	10°	15°	20°	25°	30°	35°	40°	45°	50°	55°	60°	
0.0001*	0.9890	0.9886	0.9890	0.9890	0.9892	0.9891	0.9890	0.9886	0.9885	0.9883	0.9879	0.9879	0.9879	
0.0002*	0.9848	0.9847	0.9846	0.9844	0.9844	0.9842	0.9835	0.9838	0.9833	0.9835	0.9831	0.9833	0.9831	
0.0005*	0.9756	0.9756	0.9756	0.9757	0.9759	0.9752	0.9747	0.9745	0.9741	0.9741	0.9738	0.9735	0.9734	
0.001*	0.9668	0.9662	0.9666	0.9661	0.9661	0.9656	0.9650	0.9647	0.9643	0.9644	0.9639	0.9636	0.9632	
0.002	0.9541	0.9539	0.9544	0.9530	0.9527	0.9521	0.9515	0.9513	0.9505	0.9504	0.9500	0.9497	0.9491	
0.005	0.9303	0.9300	0.9300	0.9297	0.9294	0.9285	0.9275	0.9268	0.9265	0.9261	0.9250	0.9240	0.9235	
0.01	0.9065	0.9056	0.9055	0.9055	0.9052	0.9048	0.9034	0.9025	0.9016	0.9008	0.9000	0.8990	0.8987	
0.02	0.8774	0.8768	0.8773	0.8770	0.8768	0.8755	0.8741	0.8731	0.8715	0.8704	0.8690	0.8680	0.8666	
0.05	0.8346	0.8344	0.8338	0.8329	0.8317	0.8304	0.8285	0.8265	0.8246	0.8232	0.8211	0.8195	0.8168	
0.1	0.8027	0.8023	0.8016	0.8000	0.7985	0.7964	0.7940	0.7918	0.7891	0.7872	0.7850	0.7829	0.7813	
0.2	0.7756	0.7756	0.7740	0.7717	0.7694	0.7667	0.7630	0.7604	0.7569	0.7538	0.7508	0.7474	0.7437	
0.5	0.7761	0.7730	0.7694	0.7658	0.7616	0.7571	0.7526	0.7477	0.7432	0.7387	0.7344	0.7292	0.7237	
1	0.8419	0.8363	0.8295	0.8229	0.8162	0.8090	0.8018	0.7942	0.7865	0.7790	0.7697	0.7628	0.7541	
1.5	0.9452	0.9365	0.9270	0.9154	0.9065	0.8962	0.8849	0.8740	0.8601	0.8517	0.8404	0.8276	0.8178	
2.	1.078	1.068	1.053	1.039	1.024	1.009	0.9929	0.9755	0.9602	0.9481	0.9327	0.9186	0.9072	
3.	1.452	1.427	1.401	1.373	1.345	1.316								
4	2.006	1.960	1.911	1.862	1.812	1.762								
a ₁	0.9998	1.0000	0.9995	0.9990	0.9982	0.9972	0.9958	0.9941	0.9922	0.9901	0.9879	0.9855	0.9832	
b ₁	0.01707	0.01742	0.01760	0.01782	0.01805	0.01817	0.01822	0.01825	0.01825	0.01815	0.01815	0.01805	0.01805	

* The results from 0.0001 to 0.001 *M* inclusive were obtained from values of *E* taken from the plots used in determining *E*⁰ by extrapolation.

† Extensive results at concentrations from 3 to 16 *M* have been obtained by Åkerlöf and Teare (4).

Harned and Ehlers, is recorded in table 3. Values of γ in dioxane-water mixtures have been compiled elsewhere (41, 42, 43, 52, 58) and, since they have a more specialized value, will not be recorded here. They will be employed, however, to illustrate certain theoretical considerations.

A. The activity coefficient as a function of the electrolyte concentration

A very good example of the effect of electrolyte concentration upon the activity coefficient of a 1-1 electrolyte is shown in figure 1, in which $\log \gamma$

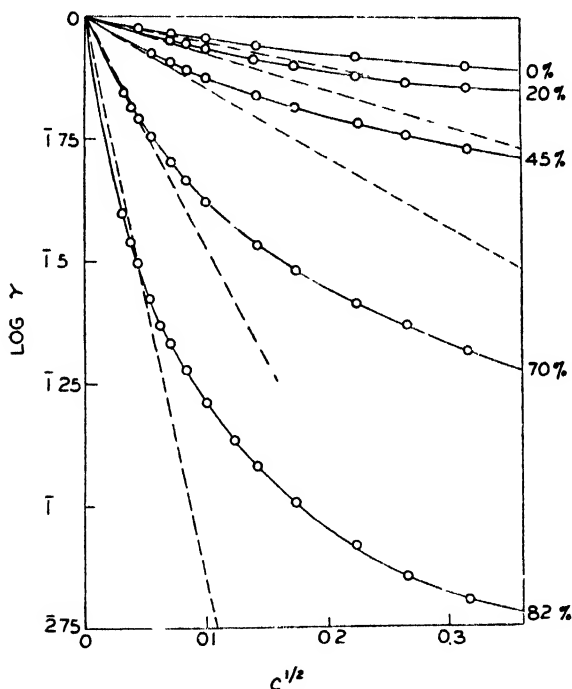


FIG. 1. Logarithm of the activity coefficient of hydrochloric acid in dioxane-water mixtures at 25°C. The straight lines (dashed) represent the limiting law. The figures on the right give the weight percentages of dioxane in the mixtures.

of hydrochloric acid in water and in dioxane-water mixtures at 25°C. is plotted against the square root of the molality, $m^{1/2}$. The straight lines represent the plots of the limiting theoretical equations for $\log f$ in these solvents,

$$\log f = -S_{(f)}\sqrt{2c} \quad (23)$$

obtained by combining equations 7 and 18. Note that in the theoretical

equation, valid at infinite dilution, c may be replaced by md_0 . At constant temperature the limiting slope, S_{∞} , for a 1-1 electrolyte is given by

$$S_{\infty} = \frac{1.283 \times 10^6}{(DT)^{3/2}} \quad (24)$$

and is a function of the dielectric constant of the solvent only. The dielectric constants of water and the 20, 45, 70, and 82 per cent dioxane-water mixtures at 25°C. are 78.54 (143), 60.79, 38.48, 17.69, and 9.53 (3), respectively. These yield 0.5065, 0.7437, 1.477, 4.738, and 11.98 for the limiting slopes of these plots, $S_{\infty}\sqrt{2}$, respectively.

The most striking characteristic of the plots in figure 1 is the general agreement in dilute solutions of the observed values with those predicted by theory. The limiting slope, $S_{\infty}\sqrt{2}$, in the 82 per cent dioxane solution is about twenty-four times as great as in water, a very large effect indeed. This striking agreement of the observed results with theory in dilute solutions shows that coulombic forces alone account for the major part of the effect even in solutions of dielectric constant as low as 9.53.

These curves also illustrate a number of characteristic behaviors of 1-1 electrolytes. Beginning with the curve at the top representing the variation of $\log \gamma$ in water, we note that all of the observed points lie above the straight line which represents the theoretical law. This fact is characteristic of all strong electrolytes. A similar result occurs for the 20 per cent and 45 per cent dioxane-water mixtures, from which we may conclude that hydrochloric acid shows little tendency to form ion pairs in these solutions. The 70 per cent dioxane mixtures show a slightly different behavior, since three observed results at the lower concentrations lie on the limiting theoretical curve, indicating some ionic association. For the 82 per cent dioxane mixtures the observed points lie somewhat below the theoretical curve at 0.001 and 0.0015 M , and considerable formation of ionic pairs may be expected.

The effect of ionic association is demonstrated in a much more pronounced manner by the curves of the molecular conductance of the acid in these solutions, shown in figure 2 (83, 124). In this case, the straight lines represent the theoretical limiting equation of Onsager,

$$\Lambda = \Lambda^0 - S_{(\Lambda)}\sqrt{2c} \quad (25)$$

in which Λ is the molecular conductance, Λ^0 its value at infinite dilution, and $S_{(\Lambda)}$ the theoretical slope. The curves may be interpreted in the same manner as those in figure 1. Since the observed results in water and in the 20 per cent and 45 per cent dioxane-water mixtures lie above the theoretical curves, hydrochloric acid is a strong electrolyte in these solvents. In the 70 per cent dioxane solutions, the observed points lie below the

theoretical plot, indicating considerable ionic association. In the 82 per cent mixtures, hydrochloric acid shows the characteristic behavior of a weak electrolyte with an ionization constant of the order of 2×10^{-4} .

It is sometimes found, even with aqueous solutions of electrolytes, that the experimental points fall *below* the theoretical limiting law, but the curve corresponding to 82 per cent dioxane-water mixtures affords so marked an example of this behavior that it is convenient at this point to mention that deviations in this direction from the limiting slope have been

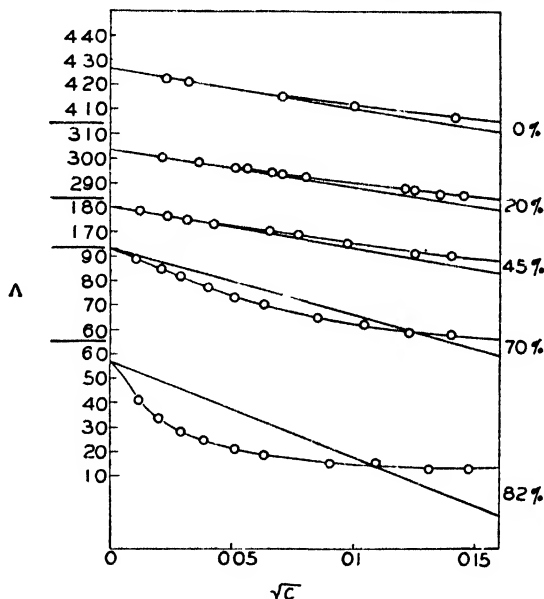


FIG. 2. Molecular conductance of hydrochloric acid in dioxane-water mixtures at 25°C. The straight lines represent the limiting Onsager conductance equation. The figures on the right give the weight percentages of dioxane in the mixtures.

examined by Gronwall, LaMer, and Sandved (19, 67). Their treatment has been applied to media of D greater than 20, but computational difficulties intervene in the case of media of lower dielectric constant. Fortunately, another treatment, due to Bjerrum (12), is applicable even in media of low dielectric constant and is easier to visualize. Bjerrum examined the probability of ion-pair formation resulting from the action of Coulomb forces and showed that this probability would have a minimum value at a critical distance, q , from any selected ion, where

$$q = \frac{\epsilon^2 |z_1 z_2|}{2DKT} \quad (26)$$

At distances greater than q the probability of ion-pair formation may be neglected, but for distances less than q the probability increases very rapidly. For a 1-1 electrolyte in aqueous solution, q is of the order of 3.5 Å. and, clearly, if the distance of closest approach of the ions, a , is greater than 3.5 Å., the electrolyte may be treated as completely dissociated. If, however, a is less than q , ion-pair formation occurs, and Bjerrum therefore treated all pairs of ions within a distance q as undissociated molecules, subject to a mass action equilibrium with the ions at distances greater than q . These were treated as fully dissociated, in conformity with the Debye-Hückel equations. Thus the dissociation constant is

$$K^{-1} = y_{12}(1 - \alpha)/y_1 y_2 \alpha^2 c \quad (27)$$

where α is the fraction of ions forming ion pairs. As a result of his calculations, Bjerrum evaluated K as

$$K^{-1} = \frac{32\pi N}{1000} q^3 Q(b) \quad (28)$$

where

$$Q(b) = \int_2^b e^{2q/r} \frac{2q^{-4}}{r} dr \quad (29)$$

and

$$b = \frac{|z_1 z_2| \epsilon^2}{a D k T} \quad (30)$$

a , the distance of closest approach, being the only quantity characteristic of a given electrolyte.

Table 4 contains values of $-\log K$ corresponding to different values of a and D , from which it may be seen that ion-pair formation is extremely sensitive to changes in the dielectric constant of the medium. The conductance data of Owen and Waters (80) give $K = 2 \times 10^{-4}$ for hydrochloric acid in an 82 per cent dioxane mixture ($D = 9.53$). From this table we see that this value of K corresponds to an a value of approximately 6 Ångströms, which is close to the value found in aqueous solutions by means of the Debye-Hückel equation.

The data in table 3 show that $\log \gamma$ at a given temperature passes through a minimum and then increases with increasing electrolyte concentration. This behavior is typical of many strong electrolytes. In order to calculate $\log \gamma$ as a function of the electrolyte concentration throughout a wide concentration range (0 to 4 M), it is necessary to introduce certain modifications of the Debye-Hückel theory. At low concen-

trations the correction due to the finite size of the ions is important. In figure 3 we have plotted $\log \gamma$ against \sqrt{m} as calculated by the limiting law (curve 1) and with the term which allows for an a value of 4.3 in equation 21 (curve 2). It will be noted that allowance for the a term raises the calculated value of the activity coefficient, but not sufficiently

TABLE 4

Dissociation constant, K , of ion pairs as a function of dielectric constant and mean ionic diameter

D	- $\log K$			
	$a = 4$	$a = 5$	$a = 6$	$a = 7$
60	-0.05			
40	0.93	0.73	0.48	
20	2.37	2.18	2.04	1.95
10	4.79	4.01	3.65	3.42
5	10.41	8.41	7.12	6.29
2.5	22.17	17.77	14.84	12.82

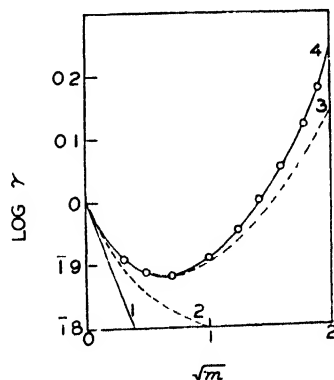


FIG. 3. Illustrating the magnitude of different terms of equation 31 for aqueous solutions of hydrochloric acid at 25°C. Curve 1, limiting law; curve 2, curve 1 + effect of a ; curve 3, curve 2 + effect of Bc term; curve 4, curve 3 + effect of $D'c^2$ term. O, experimental points.

to give a minimum in the curve. To represent the behavior at higher concentrations it is necessary to introduce terms of higher powers in c to the right of equation 21. Harned and Ehlers (44) showed that the relation

$$\log \gamma = \frac{-S_0 \sqrt{2c}}{1 + A\sqrt{2c}} + Bc + D'c^2 - \log(1 + 0.036m) \quad (31)$$

was sufficient for the accurate computation of hydrochloric acid solutions from 0° to 60°C. and from 0 to 4 *M*, an *a* value of 4.3 being used, and the empirical constants, *B* and *D'*, being expressed by:

$$B = 0.1390 - 0.000392t \quad (32)$$

$$D' = 0.0070 - 0.000033t \quad (33)$$

The equation

$$c/m = a_1 + b_1m \quad (34)$$

may be used for the conversion of molalities into molarities. Values of *a*₁ and *b*₁ are given at the bottom of table 3.

Harned and Ehlers included the terms for the Gronwall, LaMer, and Sandved extension of the theory in their calculations, but for 1-1 electrolytes in water these are very small and have been omitted here. Equation 31, with these constants, will reproduce the experimental results with an accuracy of ± 0.001 in γ over the concentration and temperature ranges indicated. We shall find it very useful in the subsequent treatment and discussion of 1-1 electrolytes.

B. The activity coefficient as a function of pressure⁴

The effect of pressure upon activity coefficients and ionic equilibria in general is of considerable interest in geological and oceanographical problems. There are sufficient data available concerning the partial molal volumes and their variation with pressure to estimate the activity coefficients of some electrolytes up to 1000 atmospheres pressure. A few examples for 1-1 electrolytes will suffice to give an idea of the magnitude of the pressure effect.

For a 1-1 electrolyte, equation 11 becomes

$$\left. \frac{\partial \ln \gamma}{\partial P} \right]_{T,m} = \frac{\bar{V}_2 - \bar{V}_2^0}{2RT} \quad (35)$$

where \bar{V}_2 and \bar{V}_2^0 are the partial molal volumes of solute at the concentration to which γ refers and at infinite dilution, respectively. If, as a first approximation, $(\bar{V}_2 - \bar{V}_2^0)$ is assumed to be independent of the pressure, integration of this equation gives

$$\log \gamma = \log \gamma_{(P=1)} + \frac{(\bar{V}_2 - \bar{V}_2^0)}{4.606RT} (P - 1) \quad (36)$$

⁴ We are indebted to Dr. Benton B. Owen of Yale University for material used in these calculations.

where γ is the activity coefficient at pressure P . Furthermore, to a good approximation

$$\bar{V}_2 - \bar{V}_2^0 = k\sqrt{c} \quad (37)$$

where k is an isothermal constant characteristic of a given electrolyte. Combination of these two equations leads to

$$\log \gamma = \log \gamma_{(P-1)} + \frac{k}{4.606RT} (P - 1)\sqrt{c} \quad (38)$$

or, at 25°C.,

$$\log \gamma = \log \gamma_{(P-1)} + 0.888 \times 10^{-5} (P - 1)k\sqrt{c} \quad (39)$$

For more accurate calculations, especially at high pressures, we must employ the expression,

$$(\bar{V}_2 - \bar{V}_2^0) = (\bar{V}_2 - \bar{V}_2^0)_{P-1} - (\bar{K}_2 - \bar{K}_2^0)(P - 1) \quad (40)$$

where \bar{K}_2 and \bar{K}_2^0 are the partial molal compressibilities of the solute. Substituting this expression for $(\bar{V}_2 - \bar{V}_2^0)$ in equation 36, and using the sufficiently close empirical approximation,

$$\bar{K}_2 - \bar{K}_2^0 = k'\sqrt{c} \quad (41)$$

we obtain at 25°C. the result:

$$\log \gamma = \log \gamma_{(P-1)} + 0.888 \times 10^{-5} k(P - 1)\sqrt{c} - 0.444 \times 10^{-5} k'(P - 1)^2\sqrt{c} \quad (42)$$

The magnitude of the pressure effect is illustrated by table 5, which contains data for the activity coefficient of hydrochloric acid at 1, 100, and 1000 atmospheres at four concentrations, and for potassium chloride, sodium chloride, and sodium hydroxide at unit concentration. At 100 atmospheres, the additional correction introduced in equation 42 is inappreciable, but at 1000 atmospheres, a comparison of the results calculated by equations 39 and 42 shows that it is no longer negligible. The last two rows contain the values of k and k' employed in the calculation. Values of k were determined from the data of Scott (122), Geffcken (18), and Gucker (20), and values of k' were obtained from Gucker and Rubin's (21) computations of the data of Lanman and Mair (69).

The activity coefficient of hydrochloric acid is not influenced greatly by a change in pressure. Even in a 2 *M* solution, only a 3 per cent change in γ is produced by a change in pressure of 1000 atmospheres. For the other electrolytes, the influence of pressure is somewhat greater. The largest effect, of 10 per cent, occurs with the activity coefficient of sodium hydroxide.

TABLE 5
Effect of pressure on activity coefficients at 25°C.

P	ACTIVITY COEFFICIENTS						
	HCl				KCl	NaCl	NaOH
	c = 0.1	c = 0.5	c = 1.0	c = 2.0	c = 1	c = 1	c = 1
1	0.797	0.757	0.807	0.991	0.605	0.6545	0.6775
100	0.7975	0.759	0.809	0.995	0.609	0.659	0.686
1000*	0.803	0.771	0.828	1.0275			
1000†	0.802	0.768	0.829	1.021	0.637	0.687	0.745
k	1.25		→		3.49	3.23	6.27
k' × 10 ⁴	4.5		→		18.6	17.1	31.3

* Equation 39.

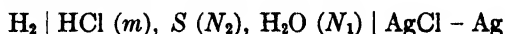
† Equation 42

TABLE 6
Standard potentials of the cells at 25°C.:
H₂ | HCl (m), Solvent (N₂), H₂O (N₁) | AgCl-Ag
N₁, N₂ = mol fractions

SOLVENT	N ₂	D	E _m ⁰	E _c ⁰	E _N ⁰	REFERENCE
Water	0	78.54	0.22239	0.22151	0.01602	(44)
Methanol-water	0.0588	74.0	0.21535	0.21431	0.01124	(57)
Methanol-water	0.1233	69.2	0.20881	0.20692	0.00710	(57)
Methanol-water	1.	31.5	-0.0101			(79)
Ethanol-water	0.0417	72.8	0.21442	0.21340	0.01123	(36)
Ethanol-water	0.0891	67.0	0.20736	0.20561	0.00763	(36)
Ethanol-water	1.	24.3	-0.0740			(142)
Glycerol-water	0.01	77	0.2196	0.2201	0.0153	(73)
Glycerol-water	0.05	72	0.2082	0.2106	0.0107	(73)
2-Propanol-water	0.0323	71.4	0.21363	0.21266	0.01095	(36)
Dioxane-water	0.0487	60.8	0.20303	0.20375	0.00554	(52)
Dioxane-water	0.1433	38.5	0.16352	0.16513	-0.02002	(52)
Dioxane-water	0.3231	17.7	0.06395	0.06584	-0.10049	(52)
Dioxane-water	0.4823	9.5	-0.0413	-0.0396	-0.19339	(52)

C. Standard potentials as a function of the composition and dielectric constant of the medium

Values of the standard potential of the cell,



in some organic solvent-water mixtures at 25°C. on the *m*-, *c*-, and *N*-scales are given in table 6. From equation 1 and the relation between electro-

motive force and free energy, it follows that the standard potentials, E_m^0 , E_c^0 , and E_N^0 , are related by

$$E_c^0 = E_m^0 + 0.1183 \log d_0 \quad (43)$$

$$E_N^0 = E_m^0 - 0.1183 \log 1000/M_{XY} \quad (44)$$

For mixed solvents, M_{XY} , is defined by

$$M_{XY} = \frac{1000}{\frac{X}{M_1} + \frac{Y}{M_2}} \quad (45)$$

where X and Y are the weight percentages, and M_1 and M_2 are the molecular weights of the two solvents, respectively (51).

In the upper portion of figure 4, E_m^0 is plotted against $1/D$ for media at high dielectric constant ($D \sim 80$ to 60). The origin of the plots on the left of the figure represents E_m^0 for pure water. None of the plots is a straight line. Further, they exhibit pronounced individual behaviors. Plots of E_c^0 , or E_N^0 , versus $1/D$ have similar characteristics.

The phenomenon of transfer of the acid from water to water-solvent mixtures can be treated conveniently in the following manner: The electromotive force of these cells at 25°C . may be represented by two fundamental equations:

$$E = E_m^0 - 0.05915 \log m_{\text{H}}m_{\text{Cl}} - 0.05915 \log \gamma_{\text{H}}\gamma_{\text{Cl}} \quad (46)$$

$$E = E_m^{0*} - 0.05915 \log m_{\text{H}}m_{\text{Cl}} - 0.05915 \log \gamma_{\text{H}}^*\gamma_{\text{Cl}}^* \quad (47)$$

In these, E_m^0 is the standard potential in water, $\gamma_{\text{H}}\gamma_{\text{Cl}}$ is the activity coefficient in any of these solutions relative to unity at infinite dilution in water, and E_m^{0*} is the standard potential in any mixture relative to unit activity coefficient, $\gamma_{\text{H}}^*\gamma_{\text{Cl}}^*$, at infinite dilution in that solvent. Combination of these equations yields

$$E_m^0 - E_m^{0*} = 0.05915 \log \frac{\gamma_{\text{H}}\gamma_{\text{Cl}}}{\gamma_{\text{H}}^*\gamma_{\text{Cl}}^*} \quad (48)$$

The superscript asterisk is employed when a transfer of an electrolyte from one medium to another is under consideration.

Further, by using the thermodynamic relationships of the reaction,



equations 46 and 47 may be combined to give

$$E_m^0 - (E_m^{0*} - 0.05915 \log a_w) = 0.05915 \log \frac{\gamma_{\text{H}_3\text{O}^+}\gamma_{\text{Cl}^-}}{\gamma_{\text{H}^+}\gamma_{\text{Cl}^-}} \quad (49)$$

where a_w is the activity of water in any mixture. Similarly,

$$E_N^0 - (E_N^{0*} = 0.05915 \log a_w) = 0.05915 \log \frac{f_{H_2O} f_{Cl}}{f_{H_2O}^* f_{Cl}^*} \quad (50)$$

The activity of pure water by convention is unity. Partial vapor pressure data indicate that, as an approximation, N_1 , the mol fraction of water, may be substituted for a_w in the solvent mixtures of high water content. This suggests the plot of $(E_N^{0*} - 0.05915 \log N_1)$ versus $1/D$, shown at

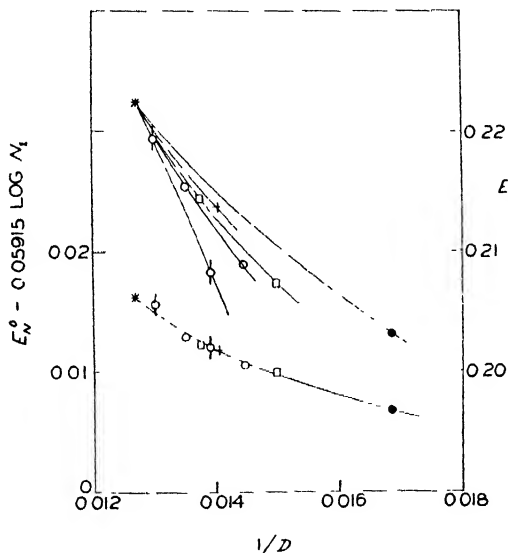


FIG 4. Plots of E_m^0 (upper curve) and $(E_N^0 - 0.05915 \log N_1)$ (lower curve) against $1/D$. *, water, O, methanol-water, □, ethanol-water; ◇, glycerol-water; +, 2-propanol-water, ●, dioxane-water.

the bottom of figure 4. In contradistinction to the result illustrated at the top of figure 4, the points for all the solvents fall very nearly on the same line. This observation may prove of considerable value in organizing data of this kind, if the result is verified by future experimental investigations. Further accurate data for the partial vapor pressure of such mixtures are highly desirable.

D. The influence of temperature upon the activity coefficient

The variation of the activity coefficient with temperature is determined by the relative partial molal heat content and heat capacity of the solute. Thus, if the activity coefficient has been determined directly over a range of temperature, equations 9 and 12 give \bar{L}_2 and \bar{J}_2 . The methods of com-

putation have been discussed by Harned and Ehlers, who calculated these thermal quantities from the data in table 3. The thermal quantities can also be determined by direct calorimetric experiments, and concordance between the two methods is valuable in giving confidence in the original activity coefficient data. In table 7 we give the three constants of a formula by means of which \bar{L}_2 and \bar{J}_2 may be expressed, the constants being calculated from E.M.F. data. In this table we also give the values of these quantities at 25°C. (columns 5 and 7) compared with the same quantities (columns 6 and 8) determined from the calorimetric measurements of Sturtevant (137) and of Gucker and Schminke (22), respectively. Con-

TABLE 7

Partial molal heat content and heat capacity of hydrochloric acid in aqueous solution

$$L_2 = L_{2(0^\circ)} + \alpha t + \beta t^2$$

(1) <i>m</i>	(2) $L_{2(0^\circ)}$	(3) α	(4) β	(5) $\bar{L}_{2(25^\circ)}$	(6) $\bar{L}_{2(25^\circ)}$	(7) $\bar{J}_{2(25^\circ)}$	(8) $\bar{J}_{2(25^\circ)}$
				<i>e.m.f.</i>	<i>cal.</i>	<i>e.m.f.</i>	<i>cal.</i>
0.005	28	0.70	0.003	47		0.85	
0.01	39	1.00	0.003	66	71	1.15	
0.02	52	1.30	0.004	87	100	1.5	
0.05	82	1.85	0.006	132	150	2.15	
0.1	113	2.50	0.008	181	203	2.9	2.4
0.2	159	3.20	0.009	245	274	3.65	3.4
0.5	272	4.70	0.011	396	431	5.25	5.3
1.0	427	6.80	0.015	606	645	7.55	7.5
1.5	615	8.20	0.019	832	860	9.15	9.2
2.0	791	10.00	0.023	1055	1056	11.15	10.6
3.0	1175	12.45	0.031	1506	1486	14.0	13.0
4.0	1604	14.70	0.040	1997		16.7	15.0

sidering the difficulties involved in both types of measurements, the agreement between the values obtained is good. Moreover, in dilute solutions the values of both \bar{L}_2 and \bar{J}_2 approach the limiting values given by equations 19 and 20. Substituting the data given in table 1, the limiting equations at 25°C. are: $\bar{L}_2 = 508\sqrt{2c}$ and $\bar{J}_2 = 9.44\sqrt{2c}$; hence at 0.01 *M*, $\bar{L}_2 = 72$ calories and $\bar{J}_2 = 1.3$ calories deg.⁻¹. These compare well with the observed values. A more general discussion of the variation of the activity coefficients of strong electrolytes with temperature will be reserved for section VII.

III. ISOPIESTIC VAPOR PRESSURE MEASUREMENTS

This method depends on the principle that in an enclosed space containing solutions of two salts, equilibrium will be reached by a distillation of solvent from the solution of higher to the solution of lower vapor pres-

sure until the concentrations are so adjusted that the vapor pressures of the two solutions are equal. The concentrations of the two isopiestic solutions, i.e., solutions of equal vapor pressure, may be obtained from the weight of salt employed and the weight of each solution after equilibrium has been attained. The successful operation (64, 74, 107, 116, 130) of the method depends upon the elimination of temperature gradients between the solutions, the requisite thermal contact being obtained by using silver containers resting on a thick copper block. Platinum and stainless steel dishes have also been found useful when working with corrosive substances, but the lower thermal conductivity of these metals makes the attainment of equilibrium a more lengthy process. The experimental results may be expressed by a plot of the isopiestic ratio, $R \equiv \nu_R m_R / \nu m$, against m , where m_R is the molality of a reference salt, such as potassium chloride, and m is the molality of the solution under investigation which has the same vapor pressure. Solutions of the salt are compared with solutions of the reference salt by a repetition of the experiment at different concentrations, and usually some twenty or thirty measurements are made to cover a concentration range from 0.1 to 4 M .

The evaluation of the activity coefficient of the salt may be made in two ways. In the first method, it is assumed that a standard curve of the osmotic coefficient of potassium chloride against the molality is known. The condition for equilibrium is that the partial molal free energy of solvent is the same in each solution, or

$$\nu m \phi = 2 m_R \phi_R \quad (51)$$

or, introducing the isopiestic ratio,

$$\phi = R \phi_R \quad (52)$$

where the subscript R refers to potassium chloride. Thus, from the standard curve and the plot of the isopiestic ratio, ϕ may be obtained at round concentrations and the activity coefficient of the salt may be calculated by the equation of Randall and White (88):

$$-\ln \gamma = h + 2 \int_0^{\sqrt{m}} h / \sqrt{m} \cdot d\sqrt{m} \quad (53)$$

where

$$h = 1 - \phi$$

In the second method, a standard curve of the activity coefficient of potassium chloride against the molality is required. This, together with the curve of the isopiestic ratio, enables the activity coefficient to be evaluated by the equation of Robinson and Sinclair (107):

$$\ln \gamma = \ln \gamma_R + \ln R + 2 \int_0^{\sqrt{a_R}} (R - 1) / \sqrt{a_R} \cdot d\sqrt{a_R} \quad (54)$$

where $a_R \equiv \gamma_R m_R$, and γ_R is the activity coefficient of potassium chloride at a concentration, m_R , isopiestic with the solution of salt at a molality, m , at which the activity coefficient of the salt is γ .

The accuracy with which activity coefficients may be obtained by this method depends on two factors: in the first place, the accuracy with which R may be determined and, secondly, the accuracy with which the set of activity coefficients, γ_R , of the reference salt is known. The first factor expresses the extent to which reproducible equilibria are attained in these experiments. Independent determinations in three laboratories show that, from careful experiments under optimum conditions, R may be determined with an accuracy of ± 0.05 per cent; this corresponds to an accuracy

TABLE 8

Osmotic and activity coefficients of sodium and potassium chlorides at 25°C.

m	ϕ_{NaCl}	γ_{NaCl}	ϕ_{KCl}	γ_{KCl}
0.1	0.932	0.778	0.926	0.769
0.2	0.925	0.734	0.913	0.717
0.3	0.921	0.710	0.906	0.687
0.5	0.922	0.682	0.900	0.650
0.7	0.927	0.668	0.898	0.626
1.0	0.938	0.658	0.899	0.605
1.5	0.959	0.659	0.906	0.585
2.0	0.986	0.671	0.915	0.575
2.5	1.017	0.692	0.927	0.572
3.0	1.050	0.720	0.941	0.573
3.5	1.085	0.753	0.955	0.576
4.0	1.122	0.792	0.970	0.582
4.5			0.985	0.590
4.81			0.996	0.595

of approximately 0.1 per cent in the determination of an activity coefficient. The method is therefore comparable in precision with the E.M.F., freezing-point, and boiling-point methods.

The uncertainty in the data for the reference salt is, unfortunately, larger, since it is a matter of considerable difficulty to determine an activity coefficient over the requisite wide range of concentration, and investigators have differed over the values to be assigned to the reference salt (65, 107, 130). The importance of obtaining a very accurate standard cannot be overestimated, and further *direct* determinations on a simple salt, such as sodium or potassium chloride, would be desirable. For the computation of results to be given later, a standard will be adopted which is based on an examination of the E.M.F. data for sodium and potassium chlorides and bromides (28, 38, 39, 40, 53), and of the direct vapor pressure data of Lovelace, Frazer, and Sease (71) on potassium chloride and of Negus (77) on sodium chloride. Table 8 gives the osmotic and activity

coefficients of potassium chloride which will be used as standards, together with similar data for sodium chloride.

Independent E.M.F. and isopiestic measurements have been made on a number of salts, and a comparison of the activity coefficients obtained by the two methods gives some information about the accuracy which may be expected. In table 9 we have made such comparisons, for a number of electrolytes, by recording the ratio of the activity coefficient calculated from E.M.F. data to that given by the isopiestic method. In the case of sodium chloride, careful measurements were made by both methods and the agreement is the best yet obtained. Up to 1 *M* the data for sodium bromide are equally satisfactory. The data for cadmium iodide, sulfuric

TABLE 9

Comparison of activity coefficients determined by electromotive force and isopiestic methods at 25°C.

<i>m</i>	RATIO $\gamma_{E.M.F.}/\gamma_{isopiestic}$					
	NaCl	NaBr	ZnSO ₄	CdI ₂	H ₂ SO ₄	SrCl ₂
0.1	1.001	1.001	1.000*	1.006	1.003	1.000*
0.2	0.998	1.001	1.000	1.000*	1.007	1.009
0.5	0.999	1.000	1.013	1.013	0.999	1.009
1.0	1.000	0.998	1.011	1.012	1.002	0.989
2.0	1.000	1.003	1.010	1.011	0.983	1.008
3.0	1.000	1.011	1.030		0.998	
4.0	1.001	0.996				
References	<div> <div>E.M.F.</div> <div>Isopiestic</div> </div>	<div>(39)</div> <div>(28, 40)</div> <div>(95)</div>	<div>(10)</div> <div>(106)</div>	<div>(8)</div> <div>(111)</div>	<div>(46)</div> <div>(98)</div>	<div>(72)</div> <div>(99)</div>

* Ratio assumed to be unity at this concentration.

acid, and strontium chloride show good agreement, although not of the order which could probably be obtained by more extensive and careful experiments. The ratios for zinc sulfate represent a poorer case, where the accuracy may have been marred by factors such as the purity of the salt.

IV. THE ACTIVITY COEFFICIENTS OF ELECTROLYTES AT 25° C.

The E.M.F. and isopiestic vapor pressure methods have been applied to a large number of strong electrolytes at 25°C. and in many cases both methods have been applied to the same electrolyte. We have reduced all the isopiestic data to the set of reference values given in table 8, and we shall now tabulate the activity coefficients of eighty-four electrolytes at 25°C., selecting those values which, in our opinion, are the more reliable

in the few cases in which the two methods yield divergent results. The tables will be followed by brief notes on the order of accuracy to be expected.

The tabulated results have been grouped as follows:

- Table 10: The chlorides, bromides, and iodides of the five alkali metals. A plot of the activity coefficients against concentration gives a series of non-intersecting curves for which the order is $\text{Li} > \text{Na} > \text{K} > \text{Rb} > \text{Cs}$ for each halide, $\text{I} > \text{Br} > \text{Cl}$ for the lithium, sodium, and potassium salts, and $\text{Cl} > \text{Br} > \text{I}$ for the rubidium and cesium salts.
- Table 11: Some alkali-metal acetates, hydroxides, and fluorides for which the order of the activity coefficient curves is $\text{Cs} > \text{Rb} > \text{K} > \text{Na} > \text{Li}$.
- Table 12: Alkali-metal nitrates and *p*-toluenesulfonates and some thalious salts. Most of these salts exhibit incomplete dissociation.
- Table 13: The results for 1-1 electrolytes are concluded with data for hydrobromic and hydriodic acids and for sodium and potassium thiocyanates.
- Table 14: Sulfuric acid.
- Table 15: A group of eighteen bivalent-metal halides.
- Table 16: Six other 1-2 electrolytes, *viz*, barium hydroxide, lithium sulfate, sodium sulfate, potassium sulfate, sodium thiosulfate, and calcium nitrate.
- Table 17: Six sulfates of bivalent metals
- Table 18: Nine chlorides of trivalent metals.
- Table 19: Three salts of higher valence type.

The following specialized notes will be useful to those interested in the order of accuracy which we would ascribe to these results:

Table 10. The data for sodium chloride and bromide, for potassium chloride, bromide, and iodide, and for cesium chloride are taken from very careful isopiestic measurements, and in each case an equally reliable set of E.M.F. measurements (28, 38, 39, 40, 53, 55) has been made. The two methods check within 0.002 in γ or less at nearly all concentrations.

The isopiestic results for lithium chloride and bromide and for sodium iodide are also confirmed by the results of E.M.F. measurements (28), but we would not attribute the same standard of accuracy to these determinations. The E.M.F. data for sodium iodide agree within 0.005 in γ up to 1 *M*, the highest concentration at which E.M.F. measurements were made, and a similar agreement is obtained for lithium chloride and lithium bromide up to 1 *M*, above which larger discrepancies occur, indicating the need for further isopiestic measurements on these two salts. Electrodes of lithium amalgam have not proved suitable for precise work (28).

The data for lithium iodide, for rubidium chloride, bromide, and iodide, and for cesium bromide and iodide depend on isopiestic data alone. The results for lithium iodide, in particular, should be investigated further.

Table 11. The values for the acetates and fluorides were evaluated from isopiestic data but, in the case of lithium, sodium, and potassium acetates

TABLE 10
*Activity coefficients of alkali-metal halides at 25°C.**

<i>m</i>	ACTIVITY COEFFICIENTS														
	LiI	LiBr	LiCl	NaI	NaBr	NaCl	KI	KBr	KCl	RbCl	RbBr	RbI	CsCl	CsBr	CsI
0.1	0.811	0.794	0.792	0.788	0.781	0.778	0.776	0.771	0.769	0.764	0.763	0.762	0.755	0.754	0.753
0.2	0.800	0.764	0.761	0.752	0.739	0.734	0.731	0.721	0.717	0.709	0.706	0.705	0.693	0.692	0.691
0.3	0.799	0.757	0.748	0.737	0.717	0.710	0.704	0.692	0.687	0.675	0.674	0.673	0.653	0.652	0.651
0.5	0.819	0.755	0.742	0.726	0.695	0.682	0.675	0.657	0.650	0.634	0.634	0.631	0.604	0.603	0.599
0.7	0.848	0.770	0.754	0.729	0.687	0.668	0.659	0.637	0.626	0.607	0.606	0.602	0.573	0.570	0.566
1.0	0.907	0.811	0.781	0.739	0.687	0.658	0.646	0.617	0.605	0.583	0.579	0.575	0.543	0.537	0.532
1.5	1.029	0.899	0.841	0.772	0.704	0.659	0.639	0.601	0.585	0.559	0.552	0.548	0.514	0.504	0.495
2.0	1.196	1.016	0.931	0.824	0.732	0.671	0.641	0.596	0.575	0.547	0.537	0.533	0.495	0.486	0.470
2.5	1.423	1.166	1.043	0.889	0.770	0.692	0.649	0.596	0.572	0.540	0.527	0.525	0.485	0.474	0.450
3.0	1.739	1.352	1.174	0.967	0.817	0.720	0.657	0.600	0.573	0.538	0.521	0.519	0.480	0.468	0.434
3.5				1.060	0.871	0.753	0.667	0.606	0.576	0.539	0.518	0.518	0.476	0.462	
4.0					0.938	0.792	0.678	0.615	0.582	0.541	0.517	0.517	0.474	0.459	
4.5							0.692		0.590	0.544	0.517	0.519	0.474	0.460	
5.0										0.547	0.518	0.520	0.476	0.460	
References	(107)	(91)	(81, 107)	(91)	(95)	(95, 116)	(111)	(95)	(95, 116)	(93)	(93)	(93)	(107)	(93)	(93)

* Seatchard and Prentiss (117, 118) have given data for ammonium chloride, ammonium bromide, ammonium iodide, lithium chloride, sodium chloride, potassium chloride, lithium bromide, sodium bromide, and potassium bromide at the freezing point. Brown and MacInnes (13) have derived the activity coefficient of sodium chloride at concentrations less than 0.1 *M* from cells with transference at 25°C.; Shedlovsky and MacInnes (127) have made similar measurements on potassium chloride up to 4 *M*. Robinson (96) has measured the isopiestic ratio, $m_{\text{KCl}}/m_{\text{NaCl}}$, in deuterium oxide.

and of sodium and potassium fluorides, results derived from freezing-point measurements give reasonable temperature coefficients of the activity coefficients, indicating that the data for these salts are at least moderately accurate.

The data for the hydroxides are from E.M.F. measurements and the results for all but lithium hydroxide should be fairly reliable. In the case of lithium hydroxide the extrapolation, as well as the results, are less accurate.

TABLE 11

*Activity coefficients of alkali-metal acetates, hydroxides, and fluorides at 25°C.**

m	ACTIVITY COEFFICIENTS										
	LiAc†	NaAc	KAc	RbAc	CsAc	LiOH	NaOH	KOH	CsOH	NaF	KF
0.05						0.803	0.818	0.824	0.831		
0.1	0.782	0.791	0.796	0.797	0.798	0.760	0.766	0.798	0.802	0.764	0.774
0.2	0.740	0.755	0.767	0.771	0.773	0.702	0.727	0.757	0.761	0.708	0.727
0.3	0.718	0.741	0.752	0.759	0.763					0.675	0.701
0.5	0.698	0.740	0.751	0.760	0.765	0.616	0.693	0.728	0.780	0.631	0.672
0.7	0.691	0.741	0.755	0.769	0.777					0.602	0.657
1.0	0.690	0.757	0.779	0.795	0.802	0.554	0.679	0.756	0.780	0.572	0.649
1.5	0.709	0.799	0.839	0.859	0.868	0.528	0.683	0.814			0.649
2.0	0.734	0.854	0.910	0.940	0.952	0.513	0.698	0.888			0.663
2.5	0.769	0.920	0.993	1.034	1.046	0.501	0.729	0.974			0.684
3.0	0.807	0.993	1.086	1.139	1.153	0.494	0.774	1.081			0.713
3.5	0.847	1.070	1.187	1.255	1.277	0.487	0.826	1.215			0.748
4.0	0.893					0.481	0.888	1.352			0.790
References	(92)	(92)	(92)	(93)	(93)	(56)	(2, 48)	(37)	(55)	(103)	(103)

* Scatchard and Prentiss (119) have given data for lithium, sodium, and potassium formates and acetates at the freezing point.

† Ac = acetate ion.

Table 12. All the values in this table were obtained from isopiestic data; again it may be shown, from a consideration of freezing-point data, that the results for lithium, sodium, and potassium nitrates are at least moderately accurate.

Table 13. The results for hydrobromic acid follow from E.M.F. measurements; for the other three electrolytes isopiestic data were used.

Table 14. The values for sulfuric acid up to 0.1 *M* were obtained by accurate E.M.F. measurements. Between 0.1 and 3 *M*, the data quoted are the mean of isopiestic (98, 116, 129) and E.M.F. (46) results which were in good agreement. Values from 5 *M* upwards obtained by both E.M.F. and direct vapor pressure measurements (123) are tabulated, since at some concentrations the agreement is not all that could be desired.

TABLE 12

Activity coefficients of alkali-metal nitrates and *p*-toluenesulfonates and of thallous salts at 25°C.*

m	ACTIVITY COEFFICIENTS										
	LiNO ₃	NaNO ₃	KNO ₃	RbNO ₃	CsNO ₃	Li \bar{S} †	Na \bar{S}	K \bar{S}	TlNO ₃	TlClO ₄	TlAc
0 1	0.788	0 758	0 733	0 730	0.729	0.773	0.764	0 760	0 701	0 730	0 748
0 2	0 751	0 702	0 659	0 656	0 651	0 729	0.708	0 701	0.605	0 652	0.684
0 3	0 737	0 664	0 607	0 603	0.598	0 698	0 672	0 662	0.544	0.599	0.643
0 5	0.728	0 615	0 542	0.534	0.526	0 664	0 624	0.607		0.527	0.588
0 7	0.731	0 583	0 494	0 484	0 475	0 642	0 592	0.562			0.552
1 0	0.746	0 548	0 441	0 429	0 419	0.621	0 551	0.509			0.513
1 5	0 783	0 509	0 378	0 365	0 354	0 595	0 502	0.438			0.472
2 0	0.840	0.481	0 327	0 319		0 574	0 460	0 387			0 444
2 5	0 903	0 457	0 293	0 284		0.565	0.428	0 349			0 422
3 0	0 973	0 438	0 266	0.256		0.563	0.403	0 318			0.405
3.5	1 052	0 423	0 244	0 235		0 566	0 385	0 294			0.390
4 0		0.410		0.216		0 573	0 368				0.377
4 5		0 398		0.200		0.584					0 365
5 0		0 388									0.354
5 5		0 380									0.345
6 0		0.373									0 336
References	(92)	(92)	(92)	(93)	(93)	(92)	(92)	(92)	(93)	(93)	(93)

* Scatchard, Prentiss, and Jones (117, 120, 121) have given data for ammonium nitrate, lithium nitrate, sodium nitrate, potassium nitrate, lithium chlorate, sodium chlorate, potassium chlorate, lithium perchlorate, sodium perchlorate, and potassium perchlorate at the freezing point.

† \bar{S} = *p*-toluenesulfonate ion; Ac = acetate ion.

TABLE 13

Activity coefficients of hydrobromic acid, hydriodic acid, sodium thiocyanate, and potassium thiocyanate at 25°C.

<i>m</i>	ACTIVITY COEFFICIENTS			
	HBr	HI	NaCNS	KCNS
0.001	0.966			
0.005	0.930			
0.01	0.906			
0 02	0.879			
0.05	0 838	0 845		
0 1	0 805	0 818	0 787	0.769
0 2	0.782	0 807	0.750	0.716
0.3		0.811	0.731	0.685
0.5	0.790	0.839	0.715	0.646
0 7		0.883	0.710	0.623
1.	0 871	0.965	0.712	0.600
1.5		1.139	0.725	0 574
2.0	1 169	1.367	0.751	0.558
2 5		1.656	0.784	0.548
3.0	1.671	2.025	0.820	0.542
3.5			0 860	0.537
4.0			0 911	0.533
4.5				0.531
5 0				0.529

Table 15. The data for the three magnesium salts, for barium bromide, and for manganese, cobalt, nickel, and cupric chlorides depend on isopiestic data only, although some support from freezing-point measurements is obtained in the cases of magnesium chloride, barium bromide, and cupric chloride. The isopiestic data from which the results for calcium chloride were obtained do not agree with E.M.F. measurements,

TABLE 14
Activity coefficient of sulfuric acid at 25°C.

<i>m</i>	γ	<i>m</i>	γ	<i>m</i>	γ
0.0005	0.885	0.02	0.453	0.7	0.140
0.0007	0.857	0.03	0.401	1.0	0.130
0.001	0.830	0.05	0.340	1.5	0.125
0.002	0.757	0.07	0.301	2.0	0.126
0.003	0.709	0.1	0.264	2.5	0.132
0.005	0.639	0.2	0.208	3.0	0.141
0.007	0.591	0.3	0.182	3.5	0.154
0.01	0.544	0.5	0.154	4.0	0.167

<i>m</i>	$\gamma^{(1)}$	$\gamma^{(2)}$
5	0.212	0.206
6	0.264	0.254
7	0.326	0.315
8	0.397	0.385
9	0.470	0.466
10	0.553	0.557
11	0.643	0.643
12	0.743	0.763
13	0.830	0.850
14	0.969	1.009
15	1.093	1.123
16	1.235	1.270
17	1.387	
17.5	1.473	

⁽¹⁾ Electromotive force (46).

⁽²⁾ Direct vapor pressure (123).

which are known to be erroneous because of the erratic behavior of the calcium amalgam electrode. In the case of strontium chloride (72) and barium chloride (72, 138), E.M.F. data are available which check the isopiestic data within approximately 0.003 in γ .

E.M.F. measurements were used to derive the data for lead chloride, zinc bromide, and zinc iodide. For zinc chloride, concordant E.M.F. and isopiestic results were available; for cadmium chloride and cadmium iodide,

TABLE 15
Activity coefficients of bivalent-metal halides at 25°C.

<i>m</i>	ACTIVITY COEFFICIENTS													
	MgCl ₂	MgBr ₂	MgI ₂	CaCl ₂ *	SrCl ₂	BaCl ₂	BaBr ₂	MnCl ₂	CoCl ₂	NiCl ₂	CuCl ₂	ZnCl ₂	ZnBr ₂	ZnI ₂
0.0005														PbCl ₂ †
0.001														0.902
0.002														0.859
0.005														0.803
0.01														0.704
0.02														0.612
0.05														0.497
0.1	0.565	0.582	0.599	0.531	0.514	0.492	0.513	0.522	0.526	0.526	0.501	0.515	0.555	0.578
0.2	0.520	0.546	0.577	0.482	0.463	0.438	0.465	0.474	0.482	0.483	0.447	0.459	0.517	0.564
0.3	0.507	0.537	0.585	0.462	0.440	0.411	0.446	0.454	0.466	0.468	0.423	0.430	0.502	0.524
0.5	0.514	0.579	0.637	0.457	0.425	0.390	0.437	0.446	0.465	0.468	0.405	0.394	0.490	0.624
0.7	0.542	0.635	0.723	0.469	0.430	0.384	0.444	0.455	0.483	0.489	0.403	0.367	0.485	0.701
1.0	0.613	0.764	0.929	0.509	0.455	0.392	0.473	0.486	0.533	0.542	0.411	0.337	0.492	0.740†
1.2	0.680	0.885	1.112	0.550	0.480	0.402	0.500	0.516	0.578	0.595	0.419	0.321	0.493	0.856
1.4	0.764	1.032	1.353	0.599	0.510	0.416	0.534	0.554	0.635	0.660	0.430	0.309	0.497	0.943
1.6	0.867	1.214	1.651	0.657	0.546	0.431	0.572	0.596	0.706	0.737	0.442	0.300	0.504	0.0506
1.8	0.986	1.440		0.726	0.587	0.450	0.616	0.637	0.785	0.826	0.454	0.294	0.511	0.0468
2.0	1.143			0.807	0.636		0.666	0.682	0.884	0.935	0.466	0.289	0.516	0.0439
2.5											0.495	0.284	0.535	0.0384
3.0											0.287	0.581		0.0351
3.5												0.632		0.0322
4.0												0.682		0.0304
5.0												0.819		0.0278
6.0												0.976		0.0263
References	(108)	(108)	(108)	(89)	(99)	(99)	(104)	(110)	(94)	(110)	(110)	(109)	(84)	(5)
													(6, 100)	(8, 111)
														(14)

* Shedlovsky and MacInnes (127) have obtained data at low concentrations from cells with transference.

† Parton, Robinson, and Metson (85) have made measurements on solutions of potassium chloride and of lead chloride.

‡ At 0.8 M.

TABLE 16

Activity coefficients of 1-2 electrolytes at 25°C.

<i>m</i>	ACTIVITY COEFFICIENTS					
	Ba(OH) ₂	Li ₂ SO ₄	Na ₂ SO ₄	K ₂ SO ₄	Na ₂ SrO ₃	Ca(NO ₃) ₂
0.005	0 773					
0.01	0.712					
0.02	0 628					
0.05	0 526	0.547	0.529	0.529		
0.1	0 441	0 468	0.445	0.441	0.455	0.480
0 2	0 370	0.399	0.365	0 361	0 382	0.421
0.3		0.362	0 321	0.317	0.340	0.391
0.5		0 321	0 268	0.264	0.292	0.360
0.7		0 299	0 234	0.233	0 263	0.344
1 0		0 280	0 203		0.236	0 334
1 2		0 273	0 188		0 224	0.332
1.5		0 268	0 171		0.212	0 334
2 0		0 269	0.153		0 200	0.343
2 5		0 278	0.143		0 197	0 359
3.0		0 293	0 138		0 201	0 379
3 5			0.136		0 209	
4.0			0 137			
References	(50)	(1, 112)	(47, 112)	(112)	(112)	(101)

TABLE 17

Activity coefficients of bivalent-metal sulfates at 25°C. (106)

<i>m</i>	ACTIVITY COEFFICIENTS					
	MgSO ₄	MnSO ₄	NiSO ₄	CuSO ₄	ZnSO ₄	CdSO ₄
0.1	(0 150)	(0 150)	(0.150)	(0.150)	(0 150)	(0 150)
0 2	0.1077	0 1056	0 1049	0 1043	0 104	0 102
0.3	0.0877	0 0850	0.0841	0.0834	0 0831	0.0815
0 4	0 0720	0 0728	0.0713	0 0708	0 0708	0 0692
0.5	0 0678	0.0643	0.0628	0.0624	0.0626	0.0609
0.7	0.0574	0.0532	0.0516	0.0515	0 0520	0.0501
1 0	0.0488	0 0441	0.0426	0 0425	0.0434	0 0411
1.5	0.0430	0.0373	0.0360		0 0378	0.0342
2.0	0 0419	0.0351	0.0343		0.0350	0.0318
2.5	0.0441	0.0353	0.0357		0.0360	0.0315
3.0	0.0495	0.0375			0.0397	0.0327
3.5		0 0416			0 0467	0 0351
4.0		0 0478				
4.25		0.0518				

TABLE 18

Activity coefficients of trivalent-metal chlorides at 25°C. (75, 76, 76a, 98)

<i>m</i>	ACTIVITY COEFFICIENTS								
	AlCl ₃	ScCl ₃	YCl ₃	LaCl ₃	CeCl ₃	PrCl ₃	NdCl ₃	SmCl ₃	EuCl ₃
0 05	(0 447)	(0 447)	(0 447)	(0 447)	(0 447)	(0 447)	(0 447)	(0 447)	(0 447)
0 1	0 389	0 384	0 382	0 383	0 380	0 380	0 381	0 385	0 385
0 2	0 353	0 341	0 337	0 337	0 333	0 333	0 333	0 340	0 342
0 3	0 351	0 333	0 326	0 323	0 319	0 319	0 318	0 329	0 329
0 5	0 384	0 355	0 338	0 328	0 324	0 322	0 322	0 333	0 334
0 7	0 449	0 403	0 373	0 354	0 350	0 346	0 348	0 363	0 367
1 0	0 621	0 523	0 465	0 424	0 420	0 413	0 418	0 442	0 448
1 2	0 814	0 647	0 559	0 493	0 488	0 482	0 488	0 520	0 527
1 4	1 087	0 813	0 686	0 587	0 577	0 573	0 581	0 623	0 637
1 6	1 508	1 033	0 858		0 696	0 686	0 703	0 761	0 781
1 8	2 170	1 326	1 091		0 862	0 834	0 862	0 941	0 973
2 0		1 706	1 417		1 067	1 033	1 079	1 182	1 237

TABLE 19

Activity coefficients of salts of higher valence type at 25°C.

<i>m</i>	ACTIVITY COEFFICIENTS		
	Al ₂ (SO ₄) ₃	In ₂ (SO ₄) ₃	K ₄ Fe(CN) ₆
0 01		0 142	
0 02		0 095	
0 03		0 071	
0 05		0 054	0 189
0 1	0 0350	0 035	0 138
0 2	0 0223	0 022	0 107
0 3	0 0174	0 017	0 088
0 4	0 0151	0 015	0 076
0 5	0 0115		0 067
0 7	0 0133		0 055
0 9			0 050
1 0	0 0176		
1 1	0 0197		
References	(93)	(61)	(93)

Measurements have also been made on the following weak electrolytes and non-electrolytes: sucrose, glycerol, urea (116); glycine (89, 131), α -alanine, α -amino-*n*-butyric acid, α -amino-*n*-valeric acid, α -aminoisobutyric acid, valine (132); β -alanine, β -amino-*n*-butyric acid, γ -aminobutyric acid, β -amino-*n*-valeric acid, γ -amino-*n*-valeric acid, ϵ -aminocaproic acid (133); proline, hydroxyproline, serine, threonine, sarcosine, and betaine (134).

E.M.F. results were used below and isopiestic results above 0.1 *M*; E.M.F. results were used for cadmium bromide but were supported by isopiestic measurements above 0.1 *M*.

Table 16. The data for barium hydroxide are derived from E.M.F. measurements; those for lithium and sodium hydroxides are from isopiestic and E.M.F. measurements which check within 0.002 in γ . In the case of potassium sulfate, freezing-point data have also been considered. The results for sodium thiosulfate and for calcium nitrate follow from isopiestic data and are supported by freezing-point data.

Table 17. The data for these 2-2 sulfates were obtained from isopiestic measurements and are all referred to a value of $\gamma = 0.150$ at 0.1 *M*. In all cases freezing-point measurements confirm these results. For copper sulfate the E.M.F. results of Nielsen and Brown (78) check these activity coefficients within less than 1 per cent; for data at lower concentrations reference may be made to the work of Wetmore and Gordon (141). The E.M.F. data of Bray (10) on zinc sulfate check the isopiestic data within 1.5 per cent up to 2.5 *M*; Cowperthwaite and LaMer (15) made measurements at lower concentrations. There is a substantial divergence between the data recorded for cadmium sulfate and the E.M.F. results of LaMer and Parks (68).

Table 18. These seven 1-3 chlorides were investigated by the isopiestic method; in the case of lanthanum chloride, independent measurements (76, 98) are in good agreement. All the results have been referred to $\gamma = 0.447$ at 0.05 *M*. Shedlovsky and MacInnes (128) have made measurements on cells with transference containing lanthanum chloride at low concentrations (see section VI).

Table 19. The results for indium sulfate follow from the E.M.F. data of Hattox and DeVries (61); the data for aluminum sulfate and potassium ferrocyanide are obtained from isopiestic measurements. It is difficult to estimate the accuracy in these cases.

It should be noted that, in the case of many of these polyvalent salts, there is considerable difficulty in assigning a reference value for the activity coefficient at the lowest concentration. This difficulty can be overcome only by precise studies of polyvalent electrolytes at low concentrations. This will require greatly improved technique or even a new mode of attack.

V. GENERAL DISCUSSION OF THE ACTIVITY COEFFICIENTS OF 1-1 ELECTROLYTES IN RELATION TO THE THEORY OF DEBYE AND HÜCKEL (17)

A. The mean distance of approach of the ions, \bar{a}

In figure 1 we have shown that the activity coefficient of hydrochloric acid varies with increasing dilution in a manner fully consistent with the

limiting laws predicted by the interionic attraction theory of Debye and Hückel. Many other examples are now available to show that the limiting equations of Debye and Hückel describe correctly the laws which electrolytes obey, the agreement becoming more exact as the dilution increases. The limiting laws, however, are strictly valid only at infinite dilution, and the factors which cause deviations at finite dilution are of great interest, as are also the theoretical interpretations of the properties of electrolytes at moderate or high concentrations.

Debye and Hückel realized that a restriction due to the finite size of the ions must be put on the Coulomb forces and introduced the parameter, \bar{a} , defined as the mean distance of approach of the ions, positive or negative. This led to an extension of the limiting law for activity coefficients of the form:

$$\begin{aligned}\log f &= \frac{-S_0\sqrt{\Gamma}}{1 + 35.57\bar{a}(DT)^{-1/2}\sqrt{\Gamma}} \\ &= \frac{S_0\sqrt{\Gamma}}{1 + K\bar{a}\sqrt{\Gamma}}\end{aligned}\quad (55)$$

The values of $K(= A/\bar{a})$ for water as solvent between 0° and 100°C. are given in the fifth column of table 1.

Later Hückel (62) extended this theory by assuming that the dielectric constant of the medium varies linearly with the concentration of the ions, thus obtaining the equation:

$$\log f = -\frac{S_0\sqrt{\Gamma}}{1 + K\bar{a}\sqrt{\Gamma}} + Bc \quad (56)$$

According to his theory, the term Bc represents the effect of salt concentration on the dielectric constant. Usually there is a lowering of the dielectric constant, corresponding to a "salting out" of the ions or a repulsive force between the ions opposite in sign to the interionic attraction effect expressed by the first term of equation 56. This effect increases the activity coefficient and, at high concentrations, this factor may be predominant.

If the parameters, \bar{a} and B , are evaluated from the experimental material, each is found to be characteristic of the electrolyte. The values of \bar{a} are always of the right order of magnitude, *viz.*, of molecular dimension, but their numerical value depends somewhat on the method of calculation employed. This is shown in table 20, which records values of \bar{a} for hydrochloric acid, potassium chloride, and sodium chloride. The values of \bar{a} , given in the first column of figures, have been calculated by the method used by MacInnes *et al.* (13, 126), using equation 21. If the linear

term of equation 56 is included in the computation, somewhat different \bar{a} values are obtained, depending on the range of concentration over which the equation is fitted to the experimental data. MacInnes *et al.* have used equation 56 between 0.005 and 0.1 *M*. Harned and Åkerlöf (33) have also used this equation between 0.1 and 3 *M*, and Harned *et al.* between 0.1 and 1 *M* (38), a better fit being obtained with the experimental data if the range of concentration is limited at 1 *M*. The values of \bar{a} obtained by these three methods of computation are recorded in table 20 in column 3. If equation 57, containing a $D'c^2$ term, is applied between 0.1 and 4 *M*, the values given in column 4 are obtained. Column 5 contains values derived by van Rysselberghe and Eisenberg, using an equation similar in form to equation 57 (see section V D, equation 66). We note that the different methods of computation do not lead to the same result, but that the values obtained are always of the right magnitude, usually somewhat

TABLE 20
Mean distance of approach of ions, \bar{a} , in Ångström units

(1) ELECTROLYTE	(2) FROM EQUATION 21	(3) FROM EQUATION 56				(4) FROM EQUATION 57	(5) FROM EQUATION 66
	0.005 to 0.1 <i>M</i>					0.1 to 4 <i>M</i>	0.1 to 4 <i>M</i>
		0.005 to 0.1 <i>M</i>	0.1 to 3 <i>M</i>	0.1 to 1 <i>M</i>			
HCl	5.6	4.6	3.6	4.2		4.3	
KCl .. .	4.1	3.7	3.4	3.8		3.95	3.2
NaCl . .	4.4	4.0	3.6	4.0		4.2	3.7

larger than the crystal dimensions, and that the \bar{a} values are in the same order, HCl > NaCl > KCl, whichever way the calculation is made.

There are two further matters of interest in connection with these \bar{a} values. In the first place, it has been found that results over a temperature range (0° to 40° or 60°C.) can be coordinated by the use of a value of \bar{a} for each electrolyte which shows no tendency to vary with temperature. This has been demonstrated by measurements on hydrochloric acid and on a number of alkali-metal salts. Secondly, the \bar{a} values for hydrochloric acid in dioxane-water mixtures have been found to be similar in magnitude to the values obtained in aqueous solution. Shedlovsky and MacInnes (126) obtained 5.6 for \bar{a} in water. By similar methods, values of 5.0, 5.4, and 5.6 were obtained for 20, 45, and 70 per cent dioxane mixtures (32, 35), and conductance data (83) led to a value of 6.0 in the 82 per cent dioxane mixture. Experimental work, therefore, reveals no substantial variation of the value of \bar{a} with either temperature or the composition of the solvent.

The application of equation 56 to a large number of electrolytes leads to some interesting results for the alkali chlorides, bromides, and iodides,

and for hydrochloric acid and hydrobromic acid. Equation 56 can be used to represent the experimental results between 0.1 and 1.0 *M* with deviations which seldom exceed 0.002 in γ , the least satisfactory fit being obtained with lithium iodide. We have found, however, that it is not possible to represent the experimental data up to 4 *M* by means of equation 56, unless we are prepared to accept deviations between observed and calculated values which are much greater than the probable experimental

TABLE 21
Constants of equations 56, 57, and 58

ELECTROLYTE	EQUATION 56		EQUATION 57				EQUATION 58		(r ₊ +r ₋)
	<i>a</i>	<i>B</i>	<i>a</i>	<i>B</i>	<i>D'</i>	Maximum deviation	<i>b</i> ₁	<i>b</i> ₂	
HI	5 0	0 197	5 5	0 1725	0 0128	0.007	0 0368	0 0014	
HBr	4.4	0 165					0 0243		
HCl	4 4	0 133	4 3	0 1292	0 00615	0 003	(Table 3)		
LiI	5 05	0 165	5 0	0 155	0 0113	0 015	0 0358	0 0009	2.77
LiBr	4 3	0.130	4 3	0.126	0 0099	0 002	0 0247	0 0002	2.56
LiCl	4 25	0 121	4 25	0.111	0 0070	0.002	0 0182		2.41
NaI	4 2	0 100	4 2	0 090	0 0058	0 008	0 0356	0 0008	3 13
NaBr	4 1	0 0687	4 2	0 0590	0 0064	0 002	0 0245		2 91
NaCl	4 0	0.0521	4 2	0.0410	0 0053	0 001	0 0183		2.76
KI	3 94	0 0462	3 95	0 0440	0.0016	0 002	0 0458	0 0014	3 50
KBr	3 84	0 0282	3 85	0 0247	0 0035	0.001	0 0345	0 0005	3.28
KCl	3 8	0 0202	3 85	0 0187	0 0034	0 001	0 0284	0.0003	3 14
RbCl	3 6	0 010	3 2	0 0235	0 0023	0 003	0 0331	0 0004	3.29
RbBr	3 55	0 010	3 2	0 0193	0 0021	0.005	0 0395	0 0008	3.43
RbI	3 5	0 0085	3 2	0 0162	0.0031	0 004	0 0508	0 0016	3 35
CsCl	3 0	0	2 5	0 0229	0 0024	0 006	0 0400	0.0008	3.46
CsBr	2 93	0	2 5	0.0162	0 0033	0 008	0 0470	0 0015	3 61
CsI	2.87	0	2 5	0 0140	0	0 006	0 0580	0 0021	3.82

errors. To secure an adequate representation of the results it is necessary to introduce into equation 56 a term containing a higher power of *c*. Thus,

$$\log f = -\frac{S_0\sqrt{\Gamma}}{1 + K\delta\sqrt{\Gamma}} + Bc + D'c^2 \quad (57)$$

We have already shown that this equation represents the data for hydrochloric acid satisfactorily, and we have investigated its application to the alkali halides, obtaining the three constants of this equation for each salt. In table 21 are given the values of *a* and *B* obtained by equation 56, *a*, *B*, and *D'* obtained by equation 57, the maximum deviation between the observed activity coefficients and those calculated by equation 57, and

the necessary data for calculating molarities from molalities at 25°C., according to the equation:

$$c/m = 0.99700 - b_1m + b_2m^2 \quad (58)$$

The last column in table 21 gives the sum of the crystallographic radii of the ions (86).

It will be observed that in nearly all cases the agreement is excellent, and is particularly good for the results in which we have most confidence. The most serious discrepancies are found with lithium iodide and sodium iodide, both of which salts require more experimental investigation.

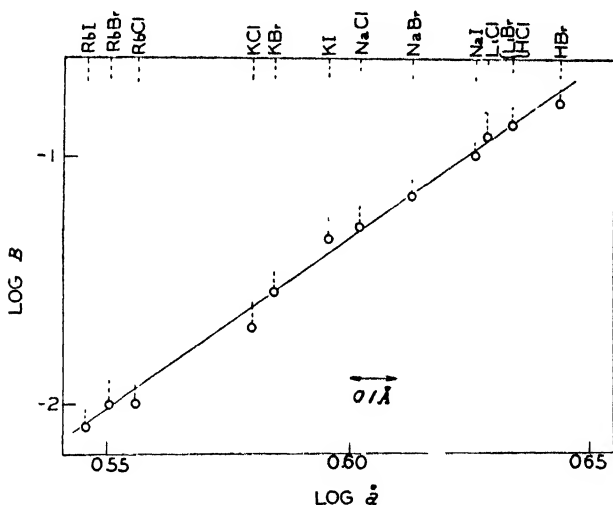


FIG. 5. $\log B$ against $\log d$ for 1-1 halides at 25°C.

There is little direct connection between the d values and the crystallographic radii but, for each series of halides, the values of d increase in the order $\text{Cs} < \text{Rb} < \text{K} < \text{Na} < \text{Li}$, which is also the order in which the crystallographic radii decrease. It will be observed from table 21 that d and B , obtained from equation 56, are in the same order and it is probable that some relation can be found between them. If this is so, then all these results can be expressed up to 1 M by a single parameter equation and constitute a single family of curves. In figure 5 we have plotted $\log d$ against $\log B$ and find that a linear relation between the two holds within the limits within which d and B can be evaluated. The equation of this straight line is

$$\log B = 14 \log d - 9.75 \quad (59)$$

which leads to the result that B is proportional to the 14^{th} power of \bar{a} , a very sensitive relation indeed if we desire to compute B from values of \bar{a} . The reverse calculation of \bar{a} from B is satisfactory. We do not consider significant the exact numerical value found for the power of \bar{a} . It merely suggests that the repulsive forces between the ions depend on a power of \bar{a} and that two equations, such as equations 56 and 59, are sufficient for the representation of the single family of curves for the alkali and hydrogen halides between 0.1 and 1.0 M .

Any relationship between \bar{a} and B , calculated from data between 0.1 and 4 M by means of equation 57, is obscured by the introduction of the third term, $D'c^2$. Nor does this constant, D' , appear to be related to B but, with so sensitive a three-parameter equation, it is doubtful if any quantitative relation can be found by induction. The tendency of \bar{a} and B to increase together, however, is still found. We shall return in section V D to a consideration of a relation similar in form to equation 57 which has some theoretical support.

B. Specific behaviors of 1-1 alkali-metal electrolytes

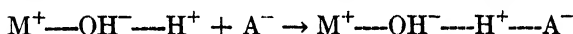
Electrolytes with "noble-gas-type cations" can be classified into three types: To the first class belong the fifteen chlorides, bromides, and iodides discussed in the preceding section. With this group we found that, if γ is plotted against \sqrt{m} , a series of regular non-intersecting curves is obtained which exhibit a wide spread at high concentrations, corresponding to large differences in the B and D' constants. Only for the cesium and possibly for the rubidium salts are the \bar{a} values (2.5 and 3.2) sufficiently low to come within the critical distance which corresponds to the ionic association of Bjerrum's theory, and even in these cases any such association which may occur must be small. The plots of γ against \sqrt{m} show only one irregularity, in that the order $\text{Cl} < \text{Br} < \text{I}$, which holds for the lithium, sodium, and potassium salts, is exactly reversed for the rubidium and cesium salts. This may be due to some ionic association, although it is difficult to understand why ionic association should be greatest for the iodides in which the anion is largest.

The consideration of these activity coefficient curves is, however, complicated if the fluorides are introduced, because now we find that the order is $\text{KF} > \text{NaF}$. The fluorides, indeed, belong to a second class of salts,—which also includes the hydroxides, formates, and acetates,—characterized by the order $\text{Cs} > \text{Rb} > \text{K} > \text{Na} > \text{Li}$, which is the reverse of that obtained for the chlorides, bromides, and iodides. This reversal in order is not directly connected with any ionic asymmetry, because the normal order $\text{K} < \text{Na}$ is found for the thiocyanates; nor is the explanation to be found in ionic association, because all the formates and acetates have \bar{a}

values considerably larger than 3.5, the critical Bjerrum diameter for aqueous solutions. Only for lithium hydroxide is a value of \bar{d} as low as 3 found, in which case ionic association may occur to some extent, as is suggested by some conductance measurements (113). This explanation cannot cover the peculiar behavior of salts, such as the acetates, with much larger values of \bar{d} . On the other hand, we note that this reversal in the order of the activity coefficient curves is found in the case of anions which are strong proton acceptors, i.e., anions derived from weak acids. It has also been noted that the dissociation of water in salt solutions is greatest in solutions of lithium salts and decreases in the order $\text{Li} > \text{Na} > \text{K} > \text{Cs}$ (31). A similar strong effect of lithium salts has been noted in the dissociation of acetic acid and of methylammonium hydroxide (54). It may, therefore, be suggested that the intense field of the small lithium ion, by strong interaction with solvent dipoles, leads to ionic hydration. The formation of a sheath of water molecules around the ion results in high values of \bar{d} , compared with crystallographic radii. This kind of ion-solvent interaction can also lead to a "localized hydrolysis" by reaction with proton acceptors. The protons in these water molecules will be repelled from the hydration sheath and will tend to form linkages with any proton acceptors, such as hydroxyl or acetate ions, in the vicinity. We may represent this tendency to distort the water molecule as follows:



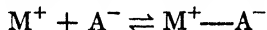
the broken lines representing the linkage due to ion-solvent molecule forces. The interaction with a proton acceptor may be represented as:



and the proton may be regarded as resonating between extreme positions on the hydroxyl group and on the proton acceptor. For thermodynamic calculations, this equilibrium is given by

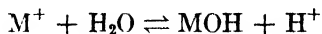


and resembles ion-pair formation of the Bjerrum type,



in that both result in a reduction in the number of ions present in the solution. This leads to a lower activity coefficient than that calculated on the assumption of complete dissociation. The two types of association are indistinguishable by thermodynamic means, but they differ in that the former occurs through the intermediate agency of a polarized water molecule. For example, the ions of lithium acetate are too large for association of the Bjerrum type; nevertheless, the field of the lithium ion is sufficiently

intense and the proton-accepting power of the acetate ion is sufficiently strong to lead to the former type of association. Ordinary hydrolysis may also occur



but this leads to no change in the number of ions.

The magnitude of the "localized hydrolysis" effect will depend on two factors. (a) the intensity with which the cation polarizes the water molecules, and (b) the strength of the anion as a proton acceptor. The latter is related to the ionization constant of the acid formed from the anion, and we are therefore led to expect that some relation will be found between this ionization constant and the dispersion of the activity coefficient curves of the alkali salts. Thus, if the anion is a very weak proton acceptor, e.g., the chloride ion, we find that the lithium salt has the highest activity coefficient. If the anion is a powerful proton acceptor, e.g., the hydroxyl ion, then the extent of association decreases in the order $Li > Na > K > Rb > Cs$, and the effect of association is large enough to reverse the order of the activity coefficient curves. For anions which are moderately strong proton acceptors we should expect, not a reversal of the order, but a closing up of the curves, and there may be a critical stage at which the curves are almost coincident. Further work should lead to some very interesting results. Although we do not have sufficient examples at present to test the hypothesis, the dispersion of the curves of the formates, acetates, and hydroxides is consistent with this mechanism. The case of the fluorides may be complicated by complex-ion formation.

A third type of behavior of 1-1 electrolytes is exemplified by the alkali nitrates, the *p*-toluenesulfonates, and the thallous salts. With the exception of the lithium salt, the alkali nitrates are almost certainly associated. This we may ascribe to the small "effective" size of the nitrate ion, without implying that the volume of the nitrate ion is abnormally small. The effective size of the ion, for the present consideration, is the distance within which the alkali-metal ion can approach one atom of the nitrate ion and, because of possible polarization effects, this may not be the same as the size of the ion estimated from crystallographic measurements. The extent to which association of the Bjerrum type occurs depends on the size of both ions, and we find that the extent of association diminishes with a decrease in the atomic weight of the cation; this order is opposite to that of the crystallographic radii, but is the order of the \bar{a} values of the chlorides, bromides, and iodides. Thus it is reasonable to believe that the effective radius determining ionic association is that of the hydrated ion and, in conformity with this, we find that the large lithium ion, even in conjunction with the "effectively" small nitrate ion, gives little or no ionic association.

Lithium nitrate has an activity coefficient curve typical of a strong electrolyte and can be described by equation 57 with $\bar{a} = 4.3$, $B = 0.0990$, and $D' = 0.00213$. In the case of potassium nitrate the existence of ionic association has been confirmed by conductance measurements, which give a dissociation constant of 1.6 (105). Some association probably occurs with the *p*-toluenesulfonates, and here again we must distinguish between the small size of the anion "effective" in collisions with the cation and the size estimated in other ways. Ionic association is pronounced with thal-
lous salts; thal-
lous nitrate, with a dissociation constant of only 0.5 (105), has an activity coefficient which lies very close to the limiting slope even up to 0.5 *M*, i.e., its apparent \bar{a} value is almost zero.

To summarize briefly, we recognize three types of 1-1 electrolytes: (a) Those with an anion of the noble-gas type, including all the hydrogen and alkali halides with the exception of the fluorides. The behavior of these electrolytes can be represented by a single family of curves, described by equation 57, with \bar{a} values which do not permit appreciable ionic association; the thiocyanates also are probably included in this category. (b) Those which exhibit reversal of the order of the activity coefficient curves, including the hydroxides, formates, acetates, and fluorides; the interpretation of their behavior is based on an hypothesis of "localized hydrolysis" leading to ionic association, not of the Bjerrum type, but by means of a water molecule as intermediary. (c) Those characterized by appreciable ionic association of the Bjerrum type due to the small "effective" size of the ions; this category is exemplified by most of the alkali nitrates and by the thal-
lous salts.

C. Guggenheim's method of computation

For the calculation of activity coefficients at concentrations up to 0.1 *M*, Guggenheim (23, 24, 25, 26) has devised a method which is sufficiently accurate for all but the most precise work. He defines a "perfect Debye-Hückel electrolyte" as one which conforms to the equation

$$\log f = -\frac{S_0\sqrt{\Gamma}}{1 + A\sqrt{\Gamma}} \quad (60)$$

which, for a 1-1 electrolyte in aqueous solution at 25°C., becomes

$$\log f = \frac{0.506\sqrt{c}}{1 + 0.3288\bar{a}\sqrt{c}} \quad (61)$$

Further, if the mean distance of approach of the ions is $\bar{a} = 3.04$, this equation reduces to the simpler form:

$$\log f = -\frac{0.506\sqrt{c}}{1 + \sqrt{c}} \quad (62)$$

Guggenheim now assumes that the specific interionic effects of the ions can be accounted for by a linear term, λc ; hence

$$\log f = -\frac{0.506\sqrt{c}}{1 + \sqrt{c}} + \lambda c \quad (63)^6$$

Guggenheim's equation permits the computation of activity coefficients up to 0.1 *M*. The activity coefficient at one intermediate concentration must be known for the calculation of the parameter λ . The success with which this equation may be applied is illustrated by the comparison (in table 22) of the activity coefficients of sodium chloride, taken from the data of Brown and MacInnes, with those calculated by equation 63, using a value of $\lambda = 0.153$.

TABLE 22

Comparison of observed activity coefficients of sodium chloride at 25°C. with those calculated by equation 63

<i>m</i>	γ_{obsd}	γ_{obsd}	<i>m</i>	$\gamma_{\text{calc'd.}}$	γ_{obsd}
0 005	0 9279	0 9283	0 04	0 8343	0 8348
0 007	0 9166	0 9171	0 05	0 8217	0 8215
0 01	0 9027	0 9032	0 06	0 8110	0 8111
0 02	0 8716	0 8724	0 08	0 7936	0 7927
0 03	0 8504	0 8509	0 10	0.7809	0 7784

The equation is very useful as an empirical method of calculation, but it is doubtful if it is sound as a theoretical equation. For, if two electrolytes are compared, then

$$\log \frac{f_1}{f_2} = (\lambda_1 - \lambda_2)c \quad (64)$$

or

$$\frac{1}{c} \log \frac{f_1}{f_2} = (\lambda_1 - \lambda_2) \quad (65)$$

⁶ This formula can be derived from equation 56 as follows:

$$\log f = \frac{0.506\sqrt{c}}{1 + 0.3288d\sqrt{c}} + Bc = -\frac{0.506\sqrt{c}}{1 + \sqrt{c}} + Bc - B'c$$

where

$$B' = \frac{0.1662(3.04 - d)}{(1 + c)(1 + 0.3288d\sqrt{c})}$$

and, up to 0.1 *M*, *B'* can be regarded for practical purposes as independent of *c* and depending only on *d*, i.e., to a good approximation $\lambda = B - B'$ is constant.

Experiments in solutions more concentrated than 0.1 *M* show that $\frac{1}{c} \log \frac{f_1}{f_2}$ always changes with concentration (29); the values in table 23, taken from the data in table 10, illustrate this.

Thus equation 65 is incompatible with the experimental data unless we admit a discontinuity in the region of 0.1 *M*. A direct experimental test of equation 63, with its implication that all ions have the same δ value, is very difficult, because only data of the highest precision in the concentration region below 0.1 *M* can be used. It is probable, however, that the experiments of MacInnes *et al.* (13, 126, 127) are of this standard, and we have applied their data to test equation 65. Figure 6 shows the experimental data expressed as $\frac{1}{c} \log \frac{f_1}{f_2}$ for the hydrochloric acid-sodium chloride and the hydrochloric acid-potassium chloride pairs, while the curves rep-

TABLE 23
Variation of $(\lambda_1 - \lambda_2)$ with concentration

<i>c</i>	0.1	0.2	0.3	0.5	0.7	1.0
$\frac{1}{c} \log \frac{\gamma_{\text{NaCl}}}{\gamma_{\text{KCl}}}$	0.061	0.054	0.050	0.046	0.042	0.037
$\frac{1}{c} \log \frac{\gamma_{\text{NaCl}}}{\gamma_{\text{KCl}}}$	0.131	0.125	0.121	0.106	0.095	0.083

resent the values of $\frac{1}{c} \log \frac{f_1}{f_2}$ calculated by equation 21, using a pair of δ values; for the upper curve the δ values are 5.5 and 4.0, for the lower curve 5.5 and 3.5. Although the position of the points is extremely sensitive to any experimental error in the original E.M.F. measurements, the general trend is towards diminishing values of $\frac{1}{c} \log \frac{f_1}{f_2}$ as the concentration increases, the decrease being of the same type as that exhibited by the curves. Thus the behavior of the experimental data is consistent with the Debye-Hückel theory for a pair of electrolytes with different δ values, and not with Guggenheim's equation which, assuming the same value of δ for all 1-1 electrolytes, predicts a constant value of $\frac{1}{c} \log \frac{f_1}{f_2}$. These considerations suggest that Guggenheim's treatment is oversimplified, although his empirical equation is well adapted for practical computation with an accuracy sufficient for most purposes. Guggenheim has given the necessary data for a large number of electrolytes at 0°C. (24).

D. The interpretation of concentrated solutions of alkali-metal halides in terms of a van der Waals covolume effect (140)

Equation 57 has been shown to give an adequate description of the activity coefficients of many 1-1 electrolytes. The first term of the equation describes the attractive forces of a Coulomb type between the ions, the restriction on these forces due to the finite size of the ions being given by the \bar{a} term in the denominator. We have already mentioned one interpretation of the Bc and $D'c^2$ terms, which Hückel ascribed to a lowering of the dielectric constant of the solution on the addition of elec-

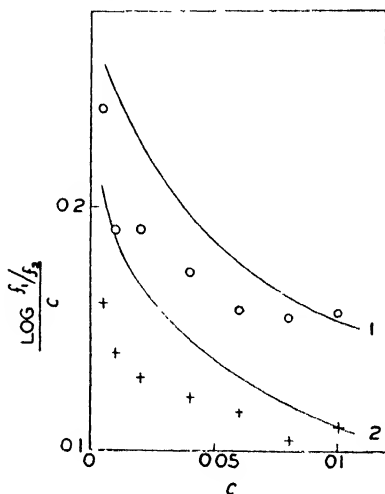


FIG. 6. Plots of $\frac{1}{c} \log \frac{f_1}{f_2}$ against c . Curve 1, calculated by equation 21, using $\bar{a} = 3.5$ and 5.5 ; curve 2, calculated by equation 21, using $\bar{a} = 4.0$ and 5.5 . \circ : 1 = HCl, 2 = KCl. $+$: 1 = HCl, 2 = NaCl.

trolyte; we shall now describe another interpretation which leads to a prediction of the values of the B and D' parameters in terms of \bar{a} . The attractive force represented by the first term of equation 57 has an analogue in van der Waals' equation; Onsager (80) suggested that the repulsive forces represented by the other terms might be ascribed to a covolume effect similar to that giving the $(v - b)$ term in van der Waals' equation. The mathematical difficulties of a treatment of this effect have only recently been overcome by Ursell (139) for the case of an imperfect gas, and van Rysselberghe and Eisenberg (140) have applied Ursell's equation to ionic solutions. They obtained the equation:

$$\log f = -\frac{S_{ij} \sqrt{2c}}{1 + K\bar{a}\sqrt{2c}} + 2.2063 \times 10^{-3} \bar{a}^3 c + 2.6269 \times 10^{-6} \bar{a}^6 c^2 \quad (66)$$

for an aqueous solution of a 1-1 electrolyte at 25°C. Thus they have succeeded in reducing equation 57 with three parameters to a one-parameter equation which gives a family of non-intersecting curves for the activity coefficients, determined solely by the distance of closest approach of the ions. Quantitatively, however, the agreement with experimental data is not good, as shown by table 24, which compares the B and D' values of three electrolytes, calculated by equation 66, with those which were used to fit the experimental data to equation 57.

van Rysselberghe and Eisenberg have succeeded, by means of their equation, in obtaining B and D' values of the right order of magnitude but by no means in quantitative agreement with the values in table 21. Nevertheless, it is significant that the calculated B and D' values are in all cases too high; in other words, equation 66 predicts too high values of the activity coefficient as a result of incorporating the covolume effect.

TABLE 24
Comparison of equations 57 and 66

ELECTROLYTE	d	B		From equation 57	From equation 66
		From equation 57	From equation 66		
HCl	4.3	0.1292	0.1754	0.00615	0.01661
KCl	3.75	0.0187	0.1163	0.0034	0.00730
CsCl	2.5	0.0229	0.0345	0.0024	0.00064

As it had previously been difficult to understand the rapid increase in the activity coefficient of electrolytes such as hydrochloric acid at high concentration, an equation which predicts even higher values is not discouraging, since the simplified treatment employed does not exclude other effects, such as ionic association, which would decrease the activity coefficient, and ion-solvent interaction.

E. Scatchard's theory of concentrated solutions (114, 115)

The most comprehensive theoretical study of concentrated solutions of strong electrolytes, particularly of the alkali-metal halides, has been made by Scatchard. The simplest case of this theoretical treatment is found if the ions of a 1-1 electrolyte can be treated as spheres; Scatchard's equation then becomes, for aqueous solutions of 1-1 electrolytes at 25°C.:

$$\log f = -\frac{0.506\sqrt{md_0}}{1 + 0.3288d\sqrt{md_0}} - f_1(a, V_+, m) + f_2(r_1, r_2, V_1, V_2, m) + f_3(V_+, m) \quad (67)$$

The first of these four terms is the limiting Debye-Hückel term, including the effect of finite ionic size. It differs from equation 61 only in the use of

md_0 in place of molarity as the unit of concentration. The next three terms are inserted to describe three effects which Scatchard regards as superimposed on the electrostatic attraction term. The term $f_1(a, V_s, m)$ is a function of a ($\equiv r_1 + r_2$), where r_1, r_2 are the ionic radii determined from crystallographic measurements, of V_s , the molal volume of the electrolyte in solution, and of m , the molality. This term expresses the correction to be applied to the charge-charge interaction consequent on the change in dielectric constant on addition of electrolyte. Whereas Hückel (62) assumes a linear variation of dielectric constant with the molarity of the electrolyte, Scatchard employs a relation found by Wyman (144) for non-electrolytic solutions. The term $f_2(r_1, r_2, V_1, V_2, m)$ contains similar variables and, in addition, the molal volume, V_s , has to be decomposed into two quantities, V_1, V_2 , characteristic of each ion. This term represents a "salting-out" effect due to a charge-solvent molecule interaction. The term $f_3(V_s, m)$ expresses a non-electrolyte molecule-molecule interaction, i.e., an interaction between ions and solvent molecules independent of the presence of charge on the solute species. The form of these functions is described in detail in the original paper (115).

For the application of the theory to computations, it is necessary to know the temperature, the dielectric constant of the solvent, the molal volumes of the ions in solution, the radius effective in "salting-out", and the effective collision diameter of the ions. In Scatchard's theory the last two quantities are obtained from crystallographic data, whereas the \bar{a} term, considered in previous equations, has to be derived from experimental data. In addition to the crystallographic radii, two constants have to be derived from two experimental activity or osmotic coefficients: one constant determines the relation of the ionic volume to the crystallographic radius, while the other gives the numerical value of the coefficient of the last term in equation 67.

The calculations by this theory agree, in general, satisfactorily with the experimental data for the alkali-metal halides given in table 10, and the theory predicts the reversal in order of activity coefficient curves found for cesium chloride, bromide, and iodide. Moreover, it predicts the higher value obtained for potassium fluoride as compared with the sodium salt.

This investigation is the most determined attempt yet made to obtain a theoretical knowledge of concentrated solutions by a detailed extension of the Debye-Hückel theory.

VI. GENERAL CONSIDERATION OF POLYVALENT ELECTROLYTES

In figure 7 are given curves for the activity coefficients of a number of 1-2 and 2-1 electrolytes. We may first direct attention to the alkaline-earth chlorides, which form a regular series, the activity coefficients diminishing with increasing atomic weight as in the case of the alkali-metal

chlorides. Equation 56 holds for barium chloride if the two parameters are given values of $\delta = 4.1$ and $B = 0.142$, the maximum deviation up to $1.2 M$ being 0.003 in γ . Equation 57 fits the data for strontium and calcium chlorides within 0.002 in γ up to a concentration of $1.2 M$, using δ values of 4.8 and 5.2 , B values of 0.100 and 0.112 , and D' values of 0.0528 and

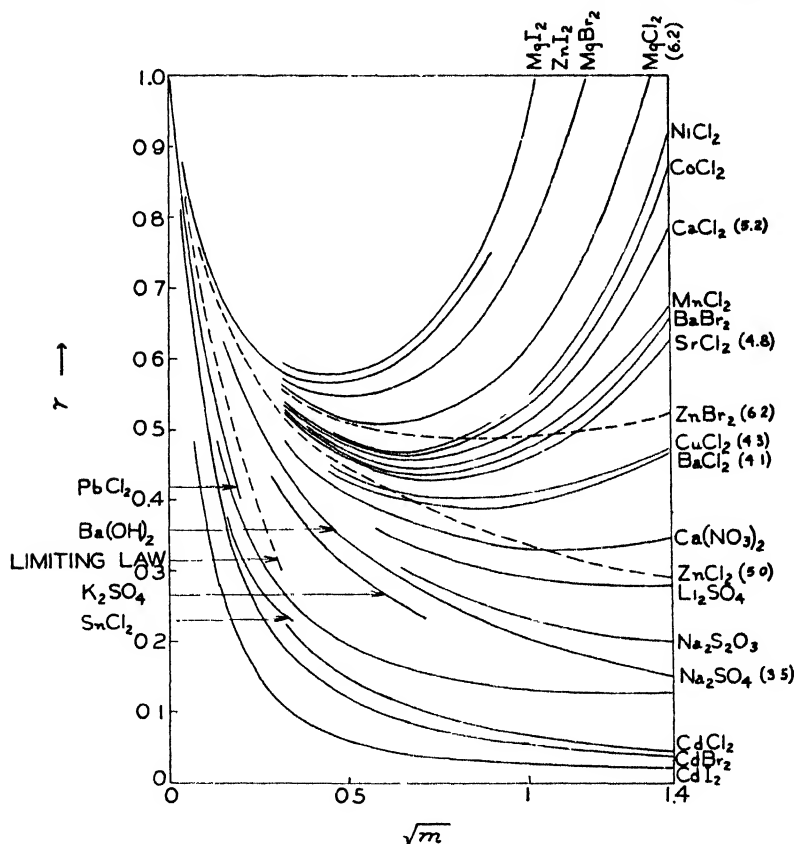


FIG. 7. Activity coefficients of 2-1 and 1-2 electrolytes. The digits on the right represent the values of δ for some of these electrolytes.⁶

0.0650 , respectively. The curves for the three magnesium halides are similar in type to those for the lithium halides and, although equation 57 does not fit so well, it can be shown that the δ values are approximately 6 .

The chlorides of metals of the transition group form an interesting

⁶Since this drawing was made, the reference value for potassium sulfate has been changed. The plot of the revised values, given in table 16, is nearly coincident with the curve for barium hydroxide and sodium sulfate.

series, with every indication that they are strong electrolytes like the alkaline-earth chlorides. The activity coefficients decrease with increasing atomic number from manganese through cobalt to nickel, the three curves being placed regularly between those of magnesium chloride and strontium chloride. In addition, we have a few unpublished measurements which indicate that the curve for ferrous chloride will lie between those of manganese chloride and cobalt chloride. With cupric chloride a change occurs; instead of lying above nickel chloride, it is found to lie very close to barium chloride, the data being represented by equation 56 with $\bar{a} = 4.3$ and $B = 0.146$.

The curve for barium bromide lies near that for strontium chloride; there is, therefore, no reversal in order such as occurs with the rubidium and cesium halides.

Zinc iodide has been investigated by means of E.M.F. measurements, with the somewhat surprising discovery that it has all the characteristics of a strong electrolyte; the E.M.F. measurements can be carried to low concentrations and an estimate made of the \bar{a} value is found to be 6.

There is therefore considerable evidence that the \bar{a} values of this group of electrolytes lie between 4 and 6; this is below the Bjerrum limit of 7 Å. for 1-2 salts, but ionic association must be small, as shown by conductance measurements on the alkaline-earth chlorides (125).

In the case of calcium nitrate, barium hydroxide, lithium sulfate, sodium sulfate, potassium sulfate, and sodium thiosulfate, we encounter electrolytes for which ionic association of the Bjerrum type can be detected by conductance measurements and the \bar{a} values, determined from the activity coefficients, are less than 4. The curves for these electrolytes spread below that of barium chloride but in all cases are above the limiting Debye slope.

For cadmium chloride, cadmium bromide, cadmium iodide, lead chloride, and stannous chloride (87), ionic association is considerable even in very dilute solution, and these systems are probably complicated by the formation of complex ions in more concentrated solution. Ionic association occurs to an extent which makes an estimate of the dissociation constants possible; a value of 0.01 is found for cadmium chloride (45), which is in agreement with that found from conductance measurements (90).

Finally, the curves for zinc chloride and zinc bromide exhibit a curious feature. In dilute solutions both salts behave as strong electrolytes, E.M.F. experiments leading to \bar{a} values of approximately 5 to 6, but above about 0.3 *M* the activity coefficient curves begin to descend in such a way as to intersect the curves for many other electrolytes; the reason for this behavior is not clear.

Little can be said about the theoretical interpretation of the data for the

2-2 metal sulfates. For such electrolytes the distance below which ionic association occurs is 14 Å. and, since Cowperthwaite and LaMer (15) found a value of 3.6 Å. for zinc sulfate, Bjerrum's theory indicates a dissociation constant of 0.003. This result receives support from the conductance measurements of Owen and Gurry (82), who obtained 0.0049 for the dissociation constant of zinc sulfate and 0.0043 for copper sulfate at 25°C. Likewise, from his examination of conductance data, Davies (16) reported a value of 0.0045 for both salts at 18°C. Finally, we may note that the activity coefficient curves of these salts spread less than those of 1-2 electrolytes. This indicates that ion-pair formation has a predominating effect, and suppresses the influence of the specific ionic character denoted by the B and D' terms of equation 57.

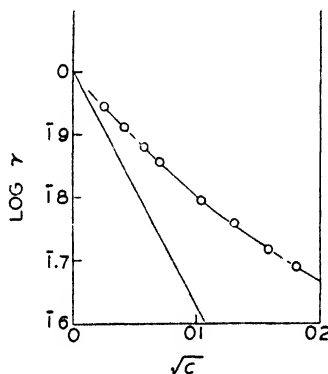


FIG. 8. Logarithm of the activity coefficient of lanthanum chloride at 25°C. The straight line represents the limiting law.

A very interesting feature of salts of higher valence type is to be found in the work of Shedlovsky and MacInnes (128) on dilute solutions of lanthanum chloride. Their activity coefficients, which extended from 0.0006 to 0.0333 M , did not approach the limiting Debye slope of 3.722 for 3-1 electrolytes. Instead their data conformed to the equation:

$$\log \gamma = -2.282\sqrt{c} + 3.20c \quad (68)$$

In figure 8, the observed values of $\log \gamma$ are plotted against \sqrt{c} . It is clear that there is no tendency for the theoretical limiting law to be approached, even in the most dilute solutions, and although ionic association might be expected to be appreciable for this salt, this effect would not act in a direction to improve the agreement of the data with the theoretical prediction. Indeed, the correction would be in the opposite

direction. The situation is rendered even more curious by the agreement of the conductance data (66) for lanthanum chloride with the Onsager prediction, whereas the transference numbers (70) are at variance with this prediction. There is thus a conflict between two closely allied irreversible processes.

VII. THE VARIATION OF THE ACTIVITY COEFFICIENT WITH TEMPERATURE

The preceding sections have been concerned with the activity coefficients of electrolytes at a single temperature, 25°C., at which a considerable amount of information is available. Not all of these electrolytes have been investigated over a range of temperature, but sodium chloride is the one example where extensive experiments have been made by different methods between 0° and 100°C. E.M.F. measurements have been made (39, 53) at temperatures between 0° and 40°C., and can be checked at the lower limit by means of freezing-point measurements (118). The determination of activity coefficients from boiling-point measurements has recently been developed to yield data of high accuracy (135, 136), and this improved technique has been used to obtain data for sodium chloride between 60° and 100°C. In addition, measurements have been made of the isopiestic ratio between potassium chloride and sodium chloride (97) over the temperature range 15° to 60°C. The problem of computing the best data from this mass of material is a difficult one, and we shall not discuss the methods of calculation here. In table 25, however, we give values of the activity coefficient of sodium chloride between 0° and 100°C. based on the above four experimental methods.

It will be noted that the data given at 25°C. are slightly different from those in table 8. This is due to the fact that the values in table 25 were derived from E.M.F. measurements only, whereas the values in table 8 were obtained from isopiestic comparisons, E.M.F., and direct vapor pressure measurements.

Sulfuric acid, as an important electrolyte, also merits a tabulation of the activity coefficients from 0° to 60°C., obtained by Harned and Hamer (46) from E.M.F. measurements. These are to be found in table 26. Apart from these two electrolytes we shall content ourselves with enumerating the electrolytes whose activity coefficients have been determined over a temperature range: hydrochloric acid (44), hydrobromic acid (49), sodium bromide (40), sodium hydroxide (2, 48), sodium sulfate (47), potassium chloride (38), potassium hydroxide, (17), barium chloride (138), zinc chloride (109), zinc iodide (5), zinc sulfate (15, 30), cadmium chloride (45), cadmium bromide (6), cadmium iodide (7), cadmium sulfate (68), and indium sulfate (61).

We shall now reverse the procedure and show how, if activity coefficients

TABLE 25
Activity coefficient of sodium chloride from 0° to 100°C.

m	ACTIVITY COEFFICIENT														
	0°	5°	10°	15°	20°	25°	30°	35°	40°	50°	60°	70°	80°	90°	100°
0.1	0.781*	0.781	0.781	0.780	0.779	0.778	0.777	0.776	0.774	0.770	0.766	0.762	0.757	0.752	0.746
0.2	0.731	0.733	0.734	0.734	0.733	0.732	0.731	0.730	0.728	(0.725)	0.721	0.717	0.711	0.705	0.698
0.5	0.671	0.675	0.677	0.678	0.679	0.679	0.679	(0.679)†	(0.678)	(0.675)	(0.671)	0.667	0.660	0.653	0.644
1.	0.638	0.644	0.649	0.652	0.654	0.656	0.657	(0.657)	0.657	(0.656)	(0.654)	0.648	0.641	0.632	0.622
1.5	0.626	0.636	0.643	0.648	0.652	0.656	0.658	(0.660)	(0.661)	(0.662)	(0.659)	(0.655)	0.646	0.638	0.629
2	0.630	0.643	0.652	0.659	0.665	0.670	0.674	(0.676)	(0.678)	(0.678)	(0.676)	0.672	0.663	0.651	0.641
2.5	0.641	0.659	0.667	0.677	0.684	0.691	0.695	(0.697)	(0.698)	(0.699)	(0.696)	(0.692)	0.685	0.674	0.659
3.	0.660	0.677	0.691	0.702	0.712	0.719	0.724	0.726	(0.728)	(0.728)	(0.726)	(0.721)	(0.712)	0.700	0.687
3.5	0.687	0.706	0.721	0.735	0.744	0.752	0.756	(0.759)	0.761	(0.762)	(0.760)	(0.758)	0.742	0.730	0.716
4.	0.717	0.740	0.757	0.772	0.783	0.791	0.797	0.800	(0.802)	(0.802)	(0.799)	(0.791)	0.777	0.763	0.746

* Referred to the value of 0.781 at 0.1 M, Seachard and Prentiss (118) obtained 0.731, 0.673, and 0.635 at 0.2, 0.5 and 1.0 M, respectively, at the freezing point.

† Values in parentheses were read from plots of the E.M.F. and boiling-point results.

are known at one temperature, thermal data may be used to calculate the activity coefficients over a temperature range. We shall assume that the relative partial molal heat of dilution may be expressed as a power series:

$$\bar{L}_2 = \bar{L}_{2(0^\circ)} + aT + bT^2 \dots \quad (70)$$

TABLE 26

Activity coefficient of sulfuric acid between 0° and 60°C. (46)

<i>m</i>	ACTIVITY COEFFICIENT						
	0°	10°	20°	30°	40°	50°	60°
0 0005	0 912	0 901	0 890	0 880	0 869	0 859	0 848
0 001	0 876	0 857	0 839	0 823	0 806	0 790	0 775
0 005	0 734	0 693	0 656	0 623	0 593	0.566	0 533
0 01	0 649	0 603	0.562	0 527	0 495	0 467	0 441
0 02	0 554	0 509	0 470	0.437	0 407	0 380	0.356
0 05	0 426	0 387	0 354	0 326	0 301	0 279	0.260
0 1	0 341	0.307	0 278	0 254	0.227	0 214	0 197
0.2	0 271	0 243	0.219	0.199	0.181	0 166	0.153
0 5	0 202	0 181	0 162	0 147	0 133	0 122	0.107
1 0	0 173	0.153	0 137	0 123	0 111	0 101	0 0922
1.5	0 167	0 147	0.131	0 117	0 106	0 0956	0 0869
2 0	0 170	0 149	0 132	0 118	0 105	0 0949	0 0859
3 0	0 201	0 173	0.151	0 132	0 117	0 104	0 0926
4.0	0 254	0 215	0 184	0.159	0 138	0 121	0 106
5 0	0 330	0.275	0 231	0 196	0 168	0 145	0 126
6.0	0.427	0.350	0.289	0 242	0 205	0.174	0 150
7.0	0 546	0 440	0.359	0 297	0 247	0 208	0 177

Experience has shown that the accuracy of the data does not require the use of powers of T higher than the second. The partial molal heat capacity relative to infinite dilution, J_2 , is given by

$$J_2 = \left. \frac{\partial \bar{L}_2}{\partial T} \right]_{P,m} = a + 2bT \quad (71)$$

and $J_{2(0^\circ)} = a$. Integrating equation 69:

$$2.303\nu RT \log \gamma = \bar{L}_{2(0^\circ)} - aT \ln T - bT^2 + I''T \quad (72)$$

where I'' is an integration constant. Let T_R be some convenient temperature at which the activity coefficient, γ_R , heat of dilution, \bar{L}_2^R , and heat capacity, J_2^R , are known. Then:

$$\log \gamma = \log \gamma_R + \frac{\bar{L}_2^R - J_2^R T_R + bT_R^2}{2.303\nu RT} - \frac{J_2^R - 2bT_R}{\nu R} \log T - \frac{bT}{2.303\nu R} + I \quad (73)$$

Fortunately, equation 73 can be simplified, because it may be assumed that \bar{J}_2 is constant in the range of temperature over which this equation will usually be employed ($0^\circ - 60^\circ\text{C}$., or somewhat higher). Any errors introduced by this assumption will not be greater than the sum of the

TABLE 27

Data for calculating the activity coefficient of sodium chloride between 0° and 100°C .

$$\log \gamma = I - \frac{A}{T} - B \log T$$

m	I	A	B
0 1	3 5083	152.06	1.2557
0 2	5 0010	221 15	1.7755
0 3	6 1564	275.49	2 1748
0.5	7.9970	364 47	2 8051
0 7	9 5163	440 34	3 3199
1 0	11 4326	535.45	3.9679
1 5	14 0912	668 38	4 8619
2 0	16 3294	779 34	5.6128

TABLE 28

Comparison of observed activity coefficients of sodium chloride with those computed by equation 75

t °C.	$(\gamma_{\text{obsd}} - \gamma_{\text{calcd.}}) \times 10^3$					
	$m = 0.1$	$m = 0.2$	$m = 0.5$	$m = 1.0$	$m = 1.5$	$m = 2.0$
0	+1	-2	-2	-1	-2	-2
10	+1	-1	-2	0	-1	0
20	0	-2	-2	-2	-3	-1
30	0	-2	-2	-3	-4	-1
40	0	-2	-2	-4	-4	-4
60	0	-1	0	0	-2	-2
80	+1	+2	+3	+1	+1	0
100	+2	+3	+4	+1	+4	+2

experimental errors of the quantities, γ_R^R , \bar{L}_2^R , and \bar{J}_2^R . Equation 73 now becomes

$$\log \gamma = \log \gamma_R + \frac{\bar{L}_2^R - \bar{J}_2^R T_R}{2.303 \nu R T} - \frac{\bar{J}_2^R}{\nu R} \log T + I' \quad (74)$$

or

$$\log \gamma = -\frac{A}{T} - \frac{B}{\log T} + I \quad (75)$$

where A , B , and I depend on data at the reference temperature, T_R . The use of this equation is limited by the paucity of reliable thermal data at high concentrations, but the equation offers a concise mode of representing a mass of activity coefficient data once the necessary thermal quantities are available.

The most recent accurate data for \bar{L}_2 and J_2 of sodium chloride have been obtained, from very low concentrations to 0.8 M , by Gulbransen and Robinson (27). From their data at 25°C. and the values of γ at 25°C. given in table 8, we have computed the constants, A , B and I , which are recorded in table 27. J_2 was found to be proportional to $m^{1/2}$ and, although an uncertainty due to extrapolation is introduced, we have employed values of J_2 up to 2 M , computed from this relation. A comparison of activity coefficients calculated by equation 75 with the values given in table 25 is made in table 28, where the deviations of the calculated from the observed values are recorded. It will be observed that the equation represents the results with accuracy from 0° to 100°C., although, as is to be expected, somewhat greater discrepancies occur at the higher concentrations.

VIII. THE THERMODYNAMIC PROPERTIES OF SODIUM CHLORIDE IN SEA WATER

In the preceding sections we have considered the variation of the activity coefficient of sodium chloride with the concentration of the solute, with temperature, and with pressure. A solution of 0.725 M sodium chloride is of particular importance because it is equivalent to "normal" sea water. We have used the data in the preceding sections to interpolate a number of thermodynamic quantities at this concentration, as follows:

$$\gamma(25^\circ) = 0.666$$

$$\phi(25^\circ) = 0.927$$

$$\text{Vapor pressure at } 25^\circ\text{C.} = 23.187 \text{ mm.}$$

$$\bar{L}_2(25^\circ) = -96 \text{ calories}$$

$$J_2(25^\circ) = 13.5 \text{ calories deg.}^{-1}$$

As a function of temperature, at a constant pressure of 1 atmosphere, the activity coefficient is given by:

$$\log \gamma = 9.7030 - 448.65/T - 3.3845 \log T \quad (76)$$

It will also be convenient to express the activity coefficient as a function of both temperature and pressure. To substitute for $(\bar{V}_2 - \bar{V}_2^0)$ in equations 36 and 40, we have used the data of Scott (122) and of Geffcken (18) to express this relative partial molal volume as:

$$\bar{V}_2 - \bar{V}_2^0 = 4.10 - 0.0729t + 0.00074t^2 \quad (77)$$

where t is the temperature in degrees Centigrade. We have also assumed that $(\bar{K}_2 - \bar{K}_2^0)$ is independent of the temperature (see section II B). This approximation should not cause any appreciable error in the final result. A combination of equations 35, 76, and 77 then gives:

$$\log \gamma = 9.7030 - 448.65/T - 3.3845 \log T + [(0.0108 - 1.93 \times 10^{-4}t + 2 \times 10^{-6}t^2)(P - 1) - 1.91 \times 10^{-6}(P - 1)^2]/T \quad (78)$$

This equation will give the activity coefficient of 0.725 M sodium chloride at a pressure of P atmospheres and at a temperature T on the absolute scale and a temperature t on the Centigrade scale. It should be applicable within the range $0^\circ < t < 50^\circ\text{C.}$ and $0 < P < 1000$.

Although this review has been concerned mainly with strong electrolytes, we may draw attention to a generalization regarding weak acids in salt solutions which should be of considerable use in oceanographical problems. This generalization⁷ is derived from three types of measurements: (a) the activity coefficient of 0.01 M hydrochloric acid in sodium chloride solutions of varying concentration; (b) the quantity $(\gamma_{\text{H}}\gamma_{\text{OH}}/a_{\text{H}_2\text{O}})^{1/2}$ pertaining to the dissociation of water in sodium chloride solutions; and (c) the quantity $(\gamma_{\text{H}}\gamma_{\text{Ac}}/\gamma_{\text{HAc}})^{1/2}$ corresponding to the dissociation of a weak monobasic acid (acetic acid) in sodium chloride solutions. Although these acids are of widely different strength, curves of the activity coefficients against the square root of the total ionic strength are found to be almost identical. At a concentration of sodium chloride equivalent to that in sea water we interpolated the following values at 25°C. : hydrochloric acid, 0.735; water, 0.719; acetic acid, 0.730, i.e., the value is to a first approximation independent of the nature of the acid. Consequently, if a problem is encountered involving the equilibria of a weak monobasic acid in sea water and the ionic activity coefficient of the acid has not been determined experimentally, a good approximation can be made by putting $(\gamma_{\text{H}}\gamma_{\text{A}}/\gamma_{\text{HA}})^{1/2} = 0.73$. This will vary little with temperature in the vicinity of 25°C.

IX. GENERAL CONSIDERATIONS

The theory of concentrated solutions of electrolytes is very difficult, and no exact quantitative treatment of all the factors involved has been approached. It has been shown in section V A that the mean distance of approach of the ions, \bar{d} , cannot be determined exactly. This is equivalent

⁷ The relevant references are as follows:

Harned, H. S., and Hawkins, J. E.: *J. Am. Chem. Soc.* **50**, 85 (1928).

Harned, H. S., and Hickey, F. C.: *J. Am. Chem. Soc.* **59**, 1284 (1937).

Harned, H. S., and Mannweiler, G. E.: *J. Am. Chem. Soc.* **57**, 1873 (1935).

Harned, H. S., and Owen, B. B.: *Chem. Rev.* **25**, 31 (1939).

¹ Harned, H. S., and Robinson, R. A.: *J. Am. Chem. Soc.* **50**, 2157 (1929).

to the statement that no observed departure from the limiting law has yet been interpreted exactly. In the paper (80) in which Onsager suggested the presence of a van der Waals covolume effect, he has presented a thoughtful critique of the theory of concentrated solutions. He has shown that the proportionality between the charge and the potential of the ion and its atmosphere, which has been used in the derivation of the limiting theoretical equations, cannot be expected to be valid in concentrated solutions. This criticism should be considered carefully in subsequent theoretical treatments of departures from the limiting law.

We have considered two interpretations of concentrated solutions (sections V D and V E). Although the treatment of the van der Waals covolume effect by van Rysselberghe and Eisenberg (140) is not found to agree accurately with experiment, it does give results of the right order of magnitude. It seems that, in some form or another, such a factor, to account for the net short-range repulsive forces between the ions, must follow from general statistical considerations.

The other method of approach, exemplified by Scatchard's extension (115) of the Debye-Hückel theory to the separate consideration of charge-charge, charge-molecule, and molecule-molecule effects, gives a reasonable explanation of the osmotic coefficients of the noble-gas-type ions. It is doubtful, however, that the linear addition of the terms representing these effects to the Debye-Hückel term can give more than a qualitative result.

None of these theories involves a detailed consideration of the structure of liquid water molecules or their orientation around ions. An extension of the theory of Bernal and Fowler (9), who have computed the heats of solution of ions in water, may prove of importance in this respect.

In investigating a state of matter as complicated as an ionic solution, it is our opinion that, from the purely scientific point of view, a knowledge of a quantity, such as the partial molal free energy, as a function of electrolyte concentration, temperature, pressure, and composition of solvent is no less important than similar investigations of the free energy of gases or single liquid substances. The experimental work now available has covered much ground but, when seen from this general point of view, there is still much to be done. The work described in section II is the most complete example of the effect of these variables on a given electrolyte, hydrochloric acid. A survey of the results so far obtained indicates that there is a need for many further studies, such as, for example, an examination of the free energy in different solvents, pressure effects as a function of temperature and solvent composition, the relationship between standard potentials and dielectric constant as suggested in figure 4, and so forth.

The activity coefficients of a large number of electrolytes at 25°C. are known, but even those most extensively studied would repay further in-

vestigation. Even in the case of salts such as sodium and potassium chloride, there is no great certainty about the data (section III).

Reference has been made in this review to the activity coefficients of nearly one hundred electrolytes at 25°C. Of these, only about twenty have been investigated over wide temperature ranges and little accurate calorimetric work has been done with concentrated solutions. Nevertheless, progress is being made in coordinating the temperature variations of activity coefficients with values of \bar{L}_2 and \bar{J}_2 determined calorimetrically. From this a greater amount of accurate information can be confidently expected in the near future. As already pointed out, this means a greater knowledge of the partial molal free energy as a function of temperature.

Besides these more "normal" investigations of a property as a function of given variables, we have now obtained abundant evidence for additional kinds of ionic interaction. One of these is illustrated by the alkali hydroxides etc. discussed in section V B. An investigation of the activity coefficients of a number of alkali-metal salts in relation to the proton-accepting power of the anion would be useful, with the object of testing the suggestion of "localized hydrolysis", made in section V B.

The data presented in table 15 and discussed in section VI are the most comprehensive yet available for 1-2 electrolytes and include many salts which hitherto have not been studied at all. The discussion of these results gives a clear idea of the relative strengths of these electrolytes. Nevertheless, very little is known about the variation of their properties with pressure, temperature, etc. For electrolytes of still higher valence type our knowledge of free-energy data is still very fragmentary.

The recent work on amino acids, referred to at the end of table 19, has revealed surprising effects on the free energy due to small changes in the carbon chain; further work on the relation between free energy and chemical constitution will be of great interest. For these systems the determination of the heat content, by measurements of the temperature coefficient of the free energy, is also important.

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VITAMIN K

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Received February 5, 1941

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I. INTRODUCTION

Owing to the methods contributed and perfected by biologists and chemists, the tempo of research in the vitamin field during the past twenty to thirty years has changed from a snail's pace to one so rapid that a worker in a particular field is often taxed to assimilate the numerous papers that appear each month. This is best illustrated by the recent developments with vitamin K. Since 1934, when Dam showed that an unknown fat-soluble dietary factor (vitamin K) is essential for the coagulation of blood in the chick, two different naturally occurring vitamins (K_1 from alfalfa and K_2 from putrefied fish meal) have been isolated, their structures determined, and one of them synthesized; numerous highly potent simple antihemorrhagic compounds have been prepared to meet requirements of physicians; and clinical investigation has made remarkable progress in the therapeutic applications of vitamin K. It is the purpose of this paper to review the story of vitamin K, which was so rapidly told in the pages of many journals during the past six years.

II. DISCOVERY OF VITAMIN K

The first observation of the symptoms which we now know are attributable to vitamin K deficiency was reported by Dam (52) in 1929. During the course of some experiments on cholesterol metabolism in the chick, he noted that chicks which had been kept on an ether-extracted diet became anemic and developed subcutaneous and intramuscular hemorrhages, and that in one chick the clotting time of the blood was prolonged. In 1930 (53), while still studying cholesterol metabolism, he observed the same hemorrhagic condition in chicks which had been kept on the ether-extracted diet supplemented with lemon juice (which contains the antiscorbutic vitamin). Since these observations were not mentioned in the summaries of these papers, it seems unlikely that Dam at that time fully appreciated the significance of this by-product of his investigation.

During a study of the fat-soluble vitamin requirements of the chick,

McFarlane (142, 143) and coworkers (1931) observed that chicks kept on a diet containing ether-extracted fish meal showed a high mortality during the third and fourth weeks, owing to hemorrhage following the insertion of wing bands. The blood of these chicks did not clot on standing overnight. There were no losses due to hemorrhage when casein or untreated fish meal was used in the diet.

Holst and Halbrook (111), in 1933, described the hemorrhagic condition and stated that it was cured by the addition of cabbage to the diet. They believed that the condition was due to a lack of vitamin C.

Continuing his work, Dam (54, 64) reported in 1934 that the hemorrhagic condition was not due to a lack of vitamins A, C, D, E, B, B₂, of fat, or of cholesterol, and in 1935 (51, 55) he suggested that the hemorrhages and prolonged clotting time were due to the lack of a new fat-soluble factor which he named vitamin K (from *Koagulations-Vitamin*). This terminology was accepted by other investigators but, following the isolation of two compounds having vitamin K activity, subscripts were added to the K for purposes of designation.

III. BIOASSAY OF ANTIHEMORRHAGIC COMPOUNDS

In every investigation directed toward the separation of an active principle (vitamins, hormones, alkaloids, etc.) from a natural source in which the concentration usually is less than 0.001 per cent, success depends upon a procedure of quantitative determination. Each step in which two fractions are obtained must be accompanied by bioassays of both fractions in order to ascertain the result of the step. Consequently, the development of a method of purification leading to the isolation of an active principle must be guided by careful and accurate bioassays. Usually this necessitates thousands of assays if the quest of the unknown is to be successful.

Frequently, as the purification proceeds and information on the chemical or physical properties of the active principle is obtained, chemical or physical methods of detection and measurement may replace the biological. For example, with vitamin K₁, Karrer (120) used the extinction coefficient at λ 248 m μ as a guide in the purification. However, such a replacement of the bioassay is usually impossible until extensive progress in the purification has been attained.

A. DEFICIENT DIETS

The discovery of a vitamin is almost invariably due to the recognition of a new syndrome which depends on the absence of an unknown substance from the diet. In the case of vitamin K, the diet used by Dam (53) had been extracted with ether. The syndrome, consisting of subcutaneous and intramuscular hemorrhages, anemia, and a prolonged clotting time, was

not relieved by the known fat-soluble vitamins (54). Almquist (21) also obtained the syndrome in experiments with chicks in which the following diet was used:

ALMQUIST'S BASAL DIET

Fish meal, ether-extracted	17.5
Brewer's yeast (dry), ether-extracted	7.5
Polished rice, ground	73.
NaCl plus small amounts of CuSO_4 and FeSO_4	1.0
Cod-liver oil	1.0

Almquist's diet has been used extensively in this country. In the experience of most laboratories, newly hatched chicks placed on this diet show a definite prolongation of clotting time within 1 week but, owing to biological variability and the mild degree of deficiency, it is necessary to continue chicks on the diet for at least another week. The chicks are suitable for assay after marked prolongation of the clotting time develops, which usually requires 2 or 3 weeks.

According to Ansbacher (28), the deficiency in a severe form can be produced in a much shorter period on a diet in which the fish meal, rice flour, and yeast are replaced by casein, heat-treated cereals, and pure vitamin B supplements, respectively.

B. BASIS OF ASSAY

The basis of all procedures of bioassay for vitamin K is the prolonged clotting time produced by a deficient diet. In normal chicks the clotting time varies from 1 to 5 min.; in chicks which show a severe form of deficiency, the blood frequently fails to clot in 3 hr. (183). Although assays may be conducted on the preventive principle, and Dam (51) and Almquist (22) in their early work used this type of procedure, practically all assays are now based on the curative principle (10, 27, 59, 66, 94, 107, 139, 167, 184, 185, 186). After maintaining a large group of chicks from the same hatch under uniform conditions of diet, etc., for about 2 weeks, the clotting times of a part of the group are determined. If the values are in excess of 60 min., the remainder of the group are suitable for the assay. The substance to be tested is administered, and after an interval blood is drawn to ascertain the response.

Many variations of the general procedure have been used. Dam and Schönheyder (65) administered the vitamin on three successive days and drew blood on the fourth day. Thayer *et al.* (185) used a similar procedure, but after Ansbacher (27) showed that the vitamin exerted its effect in a few hours, they altered their procedure to a single administration and a response period of 18 hr.

The determinations of clotting time range from the simplest, in which a wing vein is punctured, the blood caught in a crucible, and examined at room temperature until clotting occurs (51, 185), to the most refined, which include the taking of blood with a syringe or cannula, the separation of the plasma, the addition of thromboplastin, and the determination of the clotting time in a thermostat (10, 167).

Essentially, the latter procedure is the determination of prothrombin, and since a diminished concentration of this substance (167) is responsible for the prolonged clotting, assays based on its determination would theoretically be superior. However, in the purification leading to the isolation of vitamin K, assays based on the simple clotting time guided workers to the pure compound (185).

C. STANDARDS OF REFERENCE

Owing to the variability in the degree of deficiency produced by the same diet in different lots of chicks, it soon became obvious that a basic standard of reference would be necessary if accurate assays were to be obtained. Although the actual applications of the standard varied, essentially its potency was used as a base for the comparison of the potencies of the products. Dam and Glavind (59) prepared a standard spinach powder, and both Almquist (18) and Thayer (184) used a standard alfalfa extract. After the development of the field of pure antihemorrhagic compounds, Thayer *et al.* (180) suggested the adoption of 2-methyl-1,4-naphthoquinone as a standard and Dam (61) the adoption of 2-methyl-1,4-naphthohydroquinone diacetate.¹

Although the standards were necessary in the purification of the active principles of crude extracts, it appears that the adoption of the crystalline compounds as standards is not entirely satisfactory. Many factors must be considered in the comparison of a substance with a standard. Among the factors which affect the potency are: (1) the method of administration, i.e., oral, intravenous, etc.; (2) differences in the rate of absorption of the standard and the unknown; (3) the medium in which the compounds are administered; (4) differences in the rate of metabolism and excretion of the standard and the unknown; and (5) the time interval allowed for response. Consequently, it is not surprising that various investigators find different ratios of the potencies of 2-methyl-1,4-naphthoquinone and vitamin K₁.

In addition to the assay of compounds for antihemorrhagic potency, it is necessary to ascertain their toxicity before therapeutic use. The most extensive report (146) indicates that no fear should be entertained regarding the toxicity of either vitamin K₁ or 2-methyl-1,4-naphthoquinone in the dosages therapeutically effective.

¹ The solubility in water probably is so low that this compound could not be used as a standard for assays in which the substances are administered intravenously.

D. PROTHROMBIN DEFICIENCY IN MAMMALS

As every biologist knows, the choice of the experimental animal is highly important. Had Dam (53), McFarlane (143), and Almquist (23) used mammals in their experiments, it is doubtful whether vitamin K would have been discovered. After discovery of the hemorrhagic syndrome in chicks, Dam and Gilavind (60, 62) had difficulty in producing prolonged clotting times in mammals, but eventually they succeeded with both rabbits and rats. From a large number of rats, Greaves (99) finally obtained a few showing prolonged clotting time as a result of a dietary deficiency. Recently, Elliott, Isaacs, and Ivy (68) have shown that rats fed on a diet containing mineral oil (20 per cent) soon showed a marked lowering of prothrombin, and have suggested that rats prepared by this method could be used for the bioassay of antihemorrhagic compounds. Rats in which the common bile duct has been ligated may also be used for assay (93).

IV. OCCURRENCE AND DISTRIBUTION OF ANTIHEMORRHAGIC SUBSTANCES

Following the early development of bioassay procedures by Dam (51), Schønheyder (167, 168), and Almquist (3, 20), many possible sources of the vitamin were surveyed. Dam and his collaborators (49, 51, 56, 58, 65) showed that the antihemorrhagic factor is widely distributed in green leaves and vegetables. Chestnut leaves are the most potent source, but alfalfa, cabbage, spinach, grass, cauliflower, and nettle also are rich sources of the factor. Seeds (sunflower, hemp, soybean, pea, oats, wheat, and yellow corn), roots (carrots and potatoes), fruits (strawberry, hips, and ripe tomatoes), and the lower plants (moss, lichen, fungus, seaweed, and mushroom) contain only a limited amount of the vitamin. Cod-liver oil and wheat-germ oil, which are good sources of vitamins A and E, respectively, contain no vitamin K. Furthermore, Dam (55) reported that the vitamin could not be detected in beef muscle, lung, kidney, adrenals, calf brain, and thymus, but that a diet containing 20 per cent of hog liver will prevent the disease. In his early work, hog-liver fat was used as the source of the antihemorrhagic substance. Almquist and Stokstad (20, 23) reported in 1935 that the addition of 0.5 per cent of dry alfalfa to the deficient diet (see page 480) prevented the appearance of hemorrhagic symptoms, and from that time alfalfa has been one of the main sources of the vitamin.

In contrast to the distribution of carotene in carrots, vitamin K occurs in the leafy portion, but not in the roots (9). This suggested a relationship between photosynthesis and the abundance of vitamin K. Further evidence in favor of this view was furnished by Dam (58), who showed that

a much larger amount of vitamin K is found in peas grown in light than in peas grown in the dark.

In the egg the vitamin is localized in the yolk (21, 51). The reserve in the newly hatched chick is influenced by the level of vitamin K in the diet of the hen (21). The liver of the chick contains very little vitamin (21, 65), whereas, as previously noted, 20 per cent of hog liver in the diet will prevent the disease (55).

Discovery of a second antihemorrhagic factor resulted from an observation of Almquist and Stokstad (20, 23) that the protein foods used in the diets often contained a protective factor and that rice bran, fish meal, and other foods which had been stored in a moist condition developed vitamin K activity. That this production was due to the action of microorganisms was definitely concluded from Osterberg's (149) work, which showed that bacterial putrefaction of fish meal gives a highly potent antihemorrhagic product and from the report (Almquist, Pentler, and Mecchi (19)) that a large number of bacteria, including *M. tuberculosis*, *B. coli*, *B. cereus*, and *B. subtilis*, synthesize a fat-soluble antihemorrhagic factor which is not released or excreted into the culture medium. In general, microorganisms of the mold, yeast, or fungus types are inactive (9). Limburger cheese is active, whereas acidophilus milk and buttermilk contain no detectable antihemorrhagic potency.

Almquist and Stokstad (21) showed that a large amount of an ether-extractable antihemorrhagic factor is present in the fecal matter of chicks receiving a vitamin K deficient diet. Apparently this substance is synthesized by bacteria in the lower portions of the intestinal tract. McKee *et al.* (145) found appreciable quantities of vitamin K in horse, cow, sheep, hog, and human feces.

V. ISOLATION OF VITAMIN K

A. VITAMIN K₁

1. Properties of impure preparations

The early work on the purification of vitamin K was conducted chiefly by Almquist and Dam. In 1935, Dam (51, 55), working with hog-liver fat, reported that the active factor was extractable with ordinary fat solvents, was partially destroyed by cold saponification, and occurred in the non-saponifiable non-steroid fraction. Heating at 100°C. for 12 hr. did not destroy the activity. In the same year, Almquist (5, 20) showed that the factor in alfalfa occurred in the ether-soluble non-saponifiable fraction and furnished evidence which indicated that the vitamin was not an acid, ester, or base. He likewise found the vitamin to be thermostable. This observation led him (4) to introduce molecular distillation as a means

of purification. Almquist (3, 5, 8) found that the vitamin was labile toward alkali, and this observation has been confirmed repeatedly by other investigators (9, 65). In the same paper (3) Almquist reported the preparation of crude concentrates. He found that less extraneous material was extracted with the active factor from dehydrated alfalfa by hexane than by other solvents. Since this report, hexane and petroleum ether have been extensively used for preparing crude extracts.

Chlorophyll and xanthophyll were removed from hexane extracts by adsorption on activated magnesium oxide (Micron brand) and activated carbon, respectively. Other impurities were removed by partition between 90 per cent methanol and petroleum ether, the vitamin remaining in the methanol layer. The products thus obtained were subjected to molecular distillation (4, 5), the active material distilling at 120° to 140°C. at 10^{-6} mm. In 1937, Almquist (8) reported that concentrates of this type gave an active crystalline preparation when absolute methanol solutions were chilled with solid carbon dioxide. However, in the light of the present knowledge of the vitamin, it seems likely that the activity must have been due to adsorption on the surface of the crystals. Later, Almquist modified his concentration procedure somewhat, using Lloyd's reagent (7) and phosphotungstic acid (126) instead of magnesium oxide for the removal of plant pigments.

Dam (50, 63, 65) used acetone for extraction of the vitamin. He obtained partially purified products by precipitating impurities by chilling ethanol and acetone solutions and by partition between 90 per cent methanol and petroleum ether. However, differing from Almquist, he obtained the vitamin in the petroleum ether layer (65). Adsorptions on calcium carbonate and sucrose were effective in removing some of the impurities, and distillation at 115–140°C. at 10^{-3} mm. increased the potency of the product. In a study of adsorbents, he reported that the vitamin was destroyed by aluminum oxide, magnesium oxide, silicic acid, and calcium sulfate and was only weakly adsorbed by calcium carbonate and sugar. Still another adsorbent—florex—was used by Riegel (163) to effect a partial purification of vitamin K₁.

Almquist (11) reported that the vitamin could be separated from concentrates as a choleic acid. However, other investigators, using more highly purified products (37, 163), have been unable to prepare a choleic acid of the vitamin.

(a) Characterization

Although it seems likely that none of the preparations described prior to 1939 contained more than a few per cent of the vitamin and that chemical data on such products are often of little value and sometimes actually mis-

leading, a number of significant observations were made. Almquist reported the absence of sulfur, phosphorus, and nitrogen in his most active preparations (7). The potency was destroyed by reagents which react with double bonds, e.g., bromine, chromic acid, nitric acid, perbenzoic acid, ferric chloride, sulfuric acid, and hydrogen iodide (5, 6, 127). Reagents which attack hydroxyl groups, e.g., phenylisocyanate, 3,5-dinitrobenzoyl chloride, benzoyl chloride, acetic anhydride and cyanic acid,—failed to alter the activity (5, 6, 63, 127). The failure of ketonic reagents,—such as 2,4-dinitrophenylhydrazine, Girard's reagent T, and hydroxylamine—to react, indicated an absence of ketonic groups (6, 63, 127). Another important point contributed by Almquist was the discovery that the vitamin is destroyed by ultraviolet light (6). This finding and the extensive and unexplained destruction of vitamin during the course of purification led MacCorquodale *et al.* (135) to study the effect of illumination from ordinary Mazda light bulbs. They found that the purified vitamin, dissolved in benzene or ethanol, is completely destroyed after a few hours' exposure.

Thayer, MacCorquodale, Binkley, and Doisy (182) reported in 1938 that they had isolated a white crystalline compound, melting at 69°C., which after four recrystallizations possessed vitamin K activity. Subsequent work failed to confirm this report, and the error was acknowledged (37, 135). The apparent response to this compound was probably due to the bleeding of the chicks prior to the assay to determine whether they had been depleted of vitamin K (see also reference 46).

2. The product of Dam and Karrer

In March, 1939, Dam, Karrer, *et al.* (57) reported the isolation of vitamin K₁ in a pure or approximately pure form. Later (119) they stated that this product was pure vitamin K₁.² The data presented in the first paper for the chromatographically homogeneous oil are the percentages of carbon and hydrogen, the potency by bioassay, the ultraviolet absorption curve, the extinction coefficient at 248 mμ, and the effect of catalytic reduction in hexane on the ultraviolet absorption. Of these data the only accurate criterion of purity is the extinction coefficient. A comparison of the value $E_{1\text{cm}}^{1\%} = 280$ at λ 248 mμ with a large number of values obtained by other investigators (to be discussed in a later section) indicates that their product was not entirely pure.

The product of Dam and Karrer (120), the preparation of which was not described until late in 1939, was obtained from petroleum ether extracts of alfalfa by the following method: removal of the chlorophyll by adsorp-

² Dam and Karrer have given vitamin K₁ the name α-Phyllochinon (α-phyllouquinone), but thus far this name has not received wide acceptance.

tion on zinc carbonate; precipitation of impurities from petroleum ether; molecular distillation; crystallization of impurities from acetone; and, finally, chromatographic adsorption on magnesium sulfate and zinc carbonate. After seven or eight adsorptions on zinc carbonate, further adsorption failed to increase the potency of the product. A 15 to 20 per cent yield of the vitamin present in the active distillate was obtained by this process.

3. *The product of the St. Louis University group*

(a) Method of isolation

From the properties of the vitamin as determined by Dam and Almquist on the crude concentrates, it was evident that pure preparations of the vitamin could not be obtained by chemical means, e.g., removing impurities by saponification, separation of the activity into a ketonic fraction or alcoholic fraction, etc. The results of partition between various solvents and of distillation had likewise proved disappointing. As a result, investigators had turned to the possibility of chromatographic adsorption (37, 63, 74, 120, 163) as a means of purification. The method developed by Binkley *et al.* (37) led to the first isolation of vitamins K_1 and K_2 in a form of established purity. Although a large number of adsorbents (alumina, magnesium oxide, infusorial earth, Fuller's earth, supercel, magnesium oxide plus supercel, sucrose, decalso, permutit, norite, darco, nuchar, calcium sulfate, and calcium carbonate) were studied under varying conditions, the most satisfactory were permutit and decalso—two artificial zeolites used for water-softening purposes—and darco. With a modified chromatographic adsorption method, it was found that decalso and permutit are very satisfactory for the concentration of crude preparations, while darco is advantageous with preparations containing more than 10 per cent of the vitamin. The vitamin is stable toward these adsorbents; it is easily eluted; the adsorptive properties are such that a twenty- to forty-fold concentration can be obtained by a single adsorption; and the process is practically quantitative from start to finish. Decalso and permutit are suitable for handling large quantities (extract of 1000 pounds of alfalfa leaf meal) of crude extracts in one operation.

Vertical glass cylinders fitted with perforated porcelain bottoms which were covered with cotton were used in developing the process of chromatographic adsorption. For quantity production large copper percolators were used as containers for the adsorbents. The vitamin was adsorbed by allowing a petroleum ether extract of artificially dried alfalfa leaf meal to flow through the adsorbent. It was eluted by washing successively with 1:10, 1:7, and 1:5 mixtures of benzene and petroleum ether. By

using proper solvents for selective elution of the vitamin, by constantly observing the movement of the colored layers in the column, and by careful fractionation of the solvents which percolated through, a high degree of purification was attained. Three repetitions of this adsorptive process gave a reddish oil containing from 20 to 50 per cent of the vitamin. Additional adsorptions on permutit did not give any detectable purification. Further purification by adsorption on darco, followed by fractional elution, gave a lemon-yellow oil (potency 1000 units per milligram) which crystallized in yellow rosettes from acetone or ethanol at -70°C . These crystals melted at approximately -20°C . (184, 185).

Fernholz *et al.* (74), using a heat-activated calcium sulfate as an adsorbent, prepared vitamin K concentrates which had a potency comparable to that of vitamin K₁ (36).

(b) Preparation of a crystalline derivative

In order to establish definite proof of the isolation (144) of the vitamin, a crystalline derivative (136),—the diacetate of dihydrovitamin K₁ (m.p. $62-63^{\circ}\text{C}$.),—was prepared by reductive acetylation. This compound, on hydrolysis by means of the Grignard reagent and subsequent oxidation with air, gave a lemon-yellow oil identical in every respect with the original vitamin.

B. VITAMIN K₂; ISOLATION OF CRYSTALLINE PRODUCT

By application of the adsorption procedure developed for the isolation of vitamin K₁ (37), McKee *et al.* (145) isolated a different antihemorrhagic factor (36, 144) from the petroleum ether extracts of putrefied fish meal. Three adsorptions on decalso or permutit yielded a reddish yellow oil which crystallized on standing at -5°C . After several recrystallizations from an acetone-ethyl alcohol mixture or from a mixture of methyl alcohol and chloroform (1:1), a pure yellow crystalline compound melting at $53.5-54.5^{\circ}\text{C}$. was obtained. This compound had a potency of approximately 660 units per milligram (185). Evidence that the crystalline compound was actually a vitamin was based on (a) recovery of the crystals with unchanged melting point and potency after partial destruction by passage through a column of alumina, after partial oxidation with permanganate and after partial destruction during distillation, (b) the similarity of the ultraviolet absorption spectra, of the lability toward light, and of the chemical properties of the compounds isolated from alfalfa and from putrefied fish meal, (c) twenty recrystallizations from a variety of solvents without loss of potency, and (d) the preparation of several different batches having the same melting point and potency.

Analyses and molecular weight determinations indicated a formula of about $C_{40}H_{64}O_2$ (144, 145). However, degradation studies (38) later showed that the correct formula is $C_{41}H_{56}O_2$.

VI. CONSTITUTION OF VITAMIN K

A. VITAMIN K_1

On the basis of analyses for carbon and hydrogen and of molecular weight determinations, McKee *et al.* (144) proposed an empirical formula of $C_{32}H_{48}O_2$ for vitamin K_1 . Since it is not possible to determine accurately from these data the number of carbon atoms of compounds of such high molecular weight, it is not surprising that shortly thereafter (35, 137) degradative studies demonstrated that the correct formula is $C_{31}H_{46}O_2$.

1. Evidence of quinonoid structure

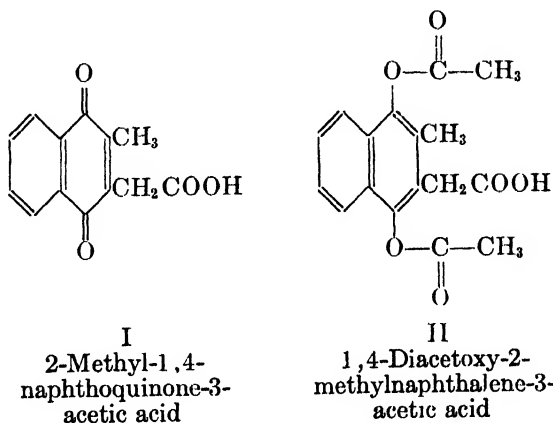
Catalytic hydrogenation (144) induced an uptake of eight atoms of hydrogen, with the formation of a colorless reduction product which upon exposure to air was converted to a yellow compound. Upon catalytic hydrogenation this yellow product absorbed two atoms of hydrogen, with the production of a colorless compound. This behavior, the absorption spectrum, the lability toward light and alkali, and the presence of two atoms of oxygen per mole led McKee *et al.* (144) to propose a quinonoid structure for vitamin K_1 . This conclusion was confirmed by the preparation of the crystalline hydroquinone diacetate, m.p. 62–63°C. Treatment of this diacetyldihydrovitamin K_1 with methylmagnesium iodide produced the hydroquinone which, upon being shaken with air, was rapidly oxidized to the vitamin.

The pure yellow color of the vitamin indicated that the substance probably belonged to the *p*-quinone series. This conclusion was supported by the discovery that, of a considerable variety of quinones, only α -naphthoquinones possessed vitamin K activity (181). Moreover, the ultra-violet absorption (71) curve indicated a close relationship to α -naphthoquinones. Since the vitamin absorbed two atoms of hydrogen in addition to the six atoms necessary for the formation of the tetrahydro derivative of the hydroquinone, it was apparent that an ethylenic linkage is present. Since the vitamin did not respond to Craven's color test, it was concluded that the vitamin is a 1,4-naphthoquinone with or without substituents in the benzenoid ring, and with hydrocarbon radicals in the 2- and 3-positions.

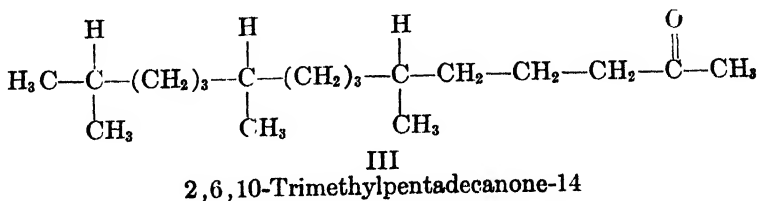
2. Oxidative degradation

Oxidation of vitamin K_1 with chromic acid resulted in the formation of a mixture of substances from which two acids were isolated. The identification of phthalic acid demonstrated that the benzenoid ring in the vitamin

is unsubstituted. The other acid, a quinone acid, was obtained as pale yellow crystals melting with decomposition at 210°C. On the basis of one analysis, it was suggested that the acid was 2-ethyl-1,4-naphthoquinone-3-acetic acid (136). However, when synthetic acids were prepared for comparison, it was found that 2-methyl-1,4-naphthoquinone-3-acetic acid had the same decomposition temperature, 210°C., whereas the 2-ethyl homolog melted with decomposition at 185°C. The methyl esters of the 2-methyl-1,4-naphthoquinone-3-acetic acid and the acid obtained from the vitamin had the same melting point (127.5–128.5°C.) and the mixture showed no depression. The quinone acid was, therefore, 2-methyl-1,4-naphthoquinone-3-acetic acid (I) (35, 137).

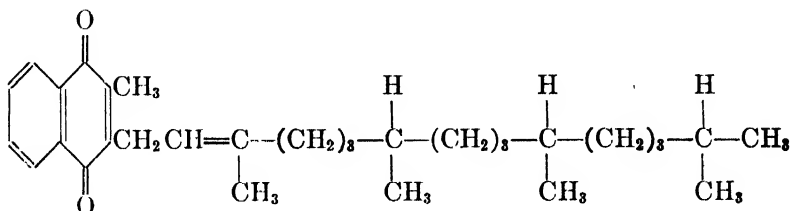


When diacetyldihydrovitamin K_1 was oxidized with chromic acid, a fairly good yield of two products was formed by cleavage at the double bond. One product, an acid having the composition $C_{17}H_{16}O_6$ and melting at 209–210°C., was identified as 1,4-diacetoxy-2-methylnaphthalene-3-acetic acid (II) by comparison of the methyl ester with a synthetic specimen. The second product was a liquid ketone. It was found that this ketone could be obtained more simply and in excellent yield by ozonolysis of the diacetate of dihydrovitamin K_1 . The ketone, isolated as the semicarbazone, proved to be identical with the semicarbazone of 2,6,10-trimethylpentadecanone-14 (III).



3. Structure of vitamin K_1

The identification of these degradation products of vitamin K_1 and its diacetyldihydro derivative clearly indicated that the constitution of the vitamin is that of 2-methyl-3-phytyl-1,4-naphthoquinone (IV).



IV
Vitamin K_1
or

2-methyl-3-phytyl-1,4-naphthoquinone

B. VITAMIN K_2

1. Evidence of quinonoid structure

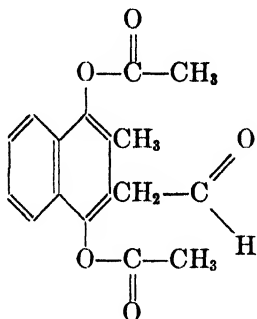
The chemical behavior of vitamin K_2 was quite similar to that of vitamin K_1 . Upon catalytic hydrogenation it absorbed 9 moles of hydrogen to produce a colorless compound which on exposure to air was oxidized to a yellow compound. This yellow compound absorbed 1 mole of hydrogen to give a colorless solution which on oxidation with air returned to the original yellow color. The yellow color, the instability to light and alkali, and the behavior in hydrogenation experiments were suggestive of the 1,4-quinones. Reductive acetylation, a reaction characteristic of quinones, gave a white crystalline diacetate of dihydrovitamin K_2 (m.p. 59.5–60°C.). Catalytic hydrogenation of this diacetate caused an uptake of 8 moles of hydrogen. The addition of 6 moles of bromine indicated the presence of six double bonds in the side chains of the molecule.

The ultraviolet absorption curves (71) for vitamins K_1 and K_2 and for 2,3-dimethyl-1,4-naphthoquinone showed a striking similarity (figure 1); likewise, the curves for the diacetates of the corresponding hydroquinones showed close agreement (figure 2). This evidence not only supported the conclusion that vitamin K_2 is a 1,4-quinone but, together with the hydrogenation data, indicated that it is a 1,4-naphthoquinone. Since, under the conditions of reduction, 3 moles of hydrogen were needed to form a tetrahydronaphthohydroquinone, the other 6 moles of hydrogen must have been used in the saturation of six double bonds in the side chains. This interpretation harmonized with the addition of 6 moles of bromine

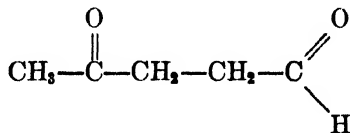
by the diacetate of dihydrovitamin K_2 . Since the vitamin did not respond to Craven's color test and did not react with maleic anhydride, it was concluded that vitamin K_2 is a 2,3-disubstituted 1,4-naphthoquinone with six double bonds, arranged without conjugation, in the side chains.

2. Oxidative degradation

Treatment of the diacetate of dihydrovitamin K_2 in glacial acetic acid with ozone, followed by decomposition of the ozonide with zinc in ether,



V
1,4-Diacetoxy-2-methylnaphthalene-
3-acetaldehyde

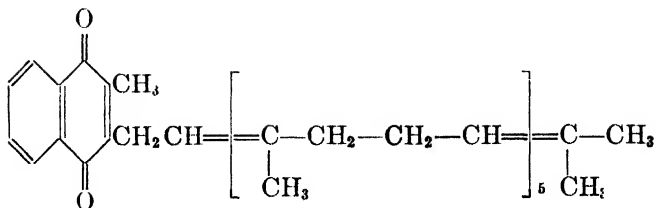


VI
Levulinolaldehyde

gave a good yield of 1,4-diacetoxy-2-methylnaphthalene-3-acetaldehyde (m.p. $115^{\circ}\text{C}.$) (V), which was characterized as the semicarbazone (m.p. $206^{\circ}\text{C}.$). Mixed melting points showed that this aldehyde was identical with 1,4-diacetoxy-2-methylnaphthalene-3-acetaldehyde, obtained from the diacetate of dihydrovitamin K_1 under the same experimental conditions. The isolation of this aldehyde demonstrated conclusively that vitamin K_2 is a 2-methyl-1,4-naphthoquinone. From the water-soluble products of the ozonization reaction, levulinolaldehyde (VI) was isolated as the bis-2,4-dinitrophenylhydrazone. On the assumption that 5 moles of levulinolaldehyde would originate from 1 mole of vitamin K_2 , a yield of 93 per cent was obtained. The third compound isolated from the ozonization reaction was acetone, which was identified as the 2,4-dinitrophenylhydrazone.

3. Structure of vitamin K_2

Since the fragments isolated from the oxidative degradation of vitamin K_2 give a total of forty-one carbon atoms, $\text{C}_{41}\text{H}_{56}\text{O}_2$ has been proposed as the correct empirical formula. The most probable arrangement of the units is expressed by structural formula VII.



VII
Vitamin K₂

VII. SYNTHESIS OF VITAMIN K₁

A. FIESER'S HYPOTHESES REGARDING THE STRUCTURE OF VITAMIN K₁

While the group of investigators at St. Louis University was approaching the problem of the structure of vitamin K₁ by degradation, followed by synthesis of the indicated structure (35, 136, 137), Fieser *et al.* (82, 83, 85) were approaching the problem from a synthetic point of view. "On the basis of Doisy's reports in the May and June Journal concerning the properties of pure vitamins K₁ and K₂, and from the observations of the Almquist, Dam-Karrer, and other groups, we advanced the hypothesis early in June that the substances are 2,3-dialkyl-1,4-naphthoquinones . . ." (84). In the July, 1939, number of the *Journal of the American Chemical Society*, Fieser, Riegel, and their collaborators (82, 86) suggested as a specific hypothesis that vitamin K₁ might be 2,6(?)-dimethyl-3-phytyl-1,4-naphthoquinone (or the 2-monomethyl compound) and that vitamin K₂ might be 2,3-difarnesyl-1,4-naphthoquinone. Fieser proceeded to test this hypothesis by an experimental attack from the synthetic approach.

B. SYNTHESSES OF VITAMIN K₁

In the September, 1939, issue of the *Journal of the American Chemical Society* the synthesis of vitamin K₁ was described by three different groups of workers. Binkley *et al.* (35) synthesized it by condensing phytol bromide with the monosodium salt of 2-methyl-1,4-naphthohydroquinone, using benzene as a solvent. The product was purified by chromatographic adsorption and distillation and identified as the diacetate of dihydrovitamin K₁. This synthetic product (138) gave the same degradative products and possessed the same potency as the natural vitamin. This same group of investigators reported that phytol condensed with 2-methyl-1,4-naphthohydroquinone in the presence of zinc chloride as a condensing agent (137).

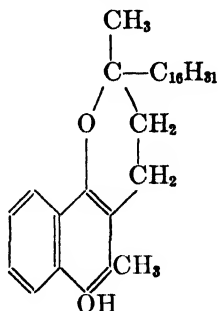
Fieser (76, 77, 78, 79, 81) found that 2-methyl-1,4-naphthohydroquinone condenses with phytol in dioxane in the presence of oxalic acid at 75°C.

The unreacted 2-methyl-1,4-naphthohydroquinone was removed with dilute alkali, and dihydrovitamin K₁ was separated on the basis of its insolubility in petroleum ether. Oxidation with silver oxide gave vitamin K₁.³ The synthetic compound was identified by its absorption spectrum, by its biological activity, and by its conversion to the diacetate of dihydrovitamin K₁. Trichloroacetic acid, phosphoric acid, acetic acid, and heat (89) were also used successfully as condensing agents.

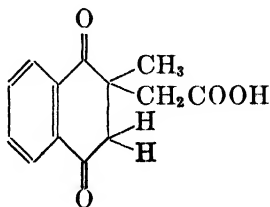
At the same time, Fieser (77) showed that natural vitamin K₁ could be extracted from partially purified concentrates by converting it to the hydroquinone, which can be extracted from petroleum ether with Claisen's alkali containing sodium hydrosulfite. After dihydrovitamin K₁ is precipitated by dilution, it is extracted with ether and purified by digestion with petroleum ether. Comparison of the diacetates of the natural and synthetic products left no doubt concerning the success of the synthetic work.

The third synthesis was reported by Almquist and Klose (15, 17), who condensed 2-methyl-1,4-naphthoquinone and phytyl bromide in petroleum ether, using zinc and acetic acid to effect the condensation. The product was purified by molecular distillation.

The synthetic procedures outlined above gave at best only moderate yields of the desired 2-methyl-3-phytyl-1,4-naphthohydroquinone and a considerable amount of a liquid by-product. In view of the formation of tocopherols by similar condensations (174), this substance was regarded as a naphthotocopherol (VIII) (86). In an extended study of the by-



VIII



IX

2-Methyl-2,3-dihydro-
1,4-naphthoquinone-
2-acetic acid

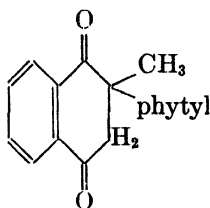
³ Frank, Hurwitz, and Seligman (96) used Fieser's synthetic vitamin K₁ in the successful treatment of patients. This was the first therapeutic use of synthetic vitamin K₁.

product, Tishler *et al.* (188) showed this hypothesis to be untenable. Naphthotocopherol was prepared by refluxing vitamin K₁ in acetic acid with stannous chloride. A study of its chemical properties and absorption spectrum showed it to be quite different from the oily by-product. Furthermore, differences in the absorption spectra of 2-methyl-1,4-naphthohydroquinone monoethyl ether and of the oily by-product indicated that the latter is not a phytol ether.

Chromic acid oxidation of the by-product gave 2-methyl-2,3-dihydro-1,4-naphthoquinone-2-acetic acid (IX) and 2,6,10-trimethylpentadecanone-14; reduction with aluminum isopropoxide yielded a secondary diol. Formation of a crystalline hydrazone likewise indicated the presence of two carbonyl groups. This evidence showed that the compound is 2-methyl-2-phytyl-2,3-dihydro-1,4-naphthoquinone (X). The substance possesses antihemorrhagic activity and can be converted in small part into vitamin K₁ by pyrolysis.

C. SYNTHESIS OF VITAMIN K₂

To date, a synthesis of vitamin K₂ has not been reported. However, degradation of the vitamin has shown conclusively that it is 2-methyl-3-(3',7',11',15',19',23'-hexamethyl-2',6',10',14',18',22'-tetracosahexenyl)-1,4-naphthoquinone (38).



X

2-Methyl-2-phytyl-2,3-dihydro-1,4-naphthoquinone

VIII. PHYSICAL AND CHEMICAL PROPERTIES OF THE NATURAL VITAMINS

Vitamin K₁ is a lemon-yellow oil at room temperature. At -70°C . it separates from acetone or ethyl alcohol in light yellow rosettes which melt at about -20°C . into an oil, plus solvent. As the temperature rises, the oil gradually passes into solution. The vitamin is soluble in the ordinary fat solvents,—ethyl alcohol, acetone, hexane, benzene, chloroform, and dioxane. It is insoluble in water and only sparingly soluble in methyl alcohol.

Vitamin K₂ is a lemon-yellow crystalline compound melting at 53.5 – 54.5°C . It may be crystallized from ethyl alcohol, acetone, or a mixture

(1:1) of methyl alcohol and chloroform. In general, it is slightly less soluble than vitamin K₁.

The diacetates of dihydrovitamin K₁ and dihydrovitamin K₂ melt at 62–63°C. and 59.5–60°C., respectively. The dibenzoate of dihydrovitamin K₁ melts at 85–86°C. (78). These derivatives may be crystallized from ethyl or methyl alcohol.

Neither vitamin rotates polarized light at a concentration of 1 per cent in ethyl alcohol.

A. ULTRAVIOLET ABSORPTION

Almquist (6), working with crude vitamin K preparations, found strong absorption in the ultraviolet, while Dam and Lewis (63) obtained no char-

TABLE 1

Ultraviolet absorption of vitamin K₁, vitamin K₂, and some 1,4-naphthoquinones

COMPOUND	λ <i>mμ</i>	LOG <i>E</i> _{molar}
Vitamin K ₁	248	4.26* (4.29)†
Vitamin K ₂	248	(4.27)†
1,4-Naphthoquinones:		
2,3-dimethyl	249	4.24* (4.29)†
2,3-diallyl	249	4.1
2-methyl-3-(β,γ,γ-trimethylallyl)	249	4.1

* Values reported by Tishler *et al.* (188), using alcohol as the solvent.

† Values reported by Ewing (unpublished data), using hexane as the solvent.

acteristic absorption with similar preparations. Dam, Karrer, *et al.* (57) and Karrer *et al.* (119, 120) reported maxima at 248, 261, 270, and 328 *mμ* for a preparation which they considered to be the pure or nearly pure vitamin. McKee *et al.* (144), in their first report on the isolation of vitamin K, found that vitamin K₁ shows maxima at 243, 248, 261, 270 and 323 *mμ* and vitamin K₂ shows maxima at 249, 261, 269, and 320 *mμ*. Whereas Dam, Karrer, *et al.* (57) and Karrer *et al.* (119, 120) found an extinction coefficient of $E_{1\%}^{1\text{cm.}} = 280$ at 248 *mμ*, McKee *et al.* reported the value 385. Since the intensity of absorption, as measured by the extinction coefficient, is an accurate index of the relative purity of different samples, the American workers concluded that the Dam-Karrer product was 70 per cent pure.⁴

⁴ In order to obtain more information on the cause of the discrepancy, specimens of the same preparations of natural and synthetic vitamin K₁ were supplied to both Karrer and Ewing for the determination of the extinction coefficient. The values reported for $E_{1\%}^{1\text{cm.}}$ at λ 248 *mμ* by Ewing were 421 for the natural vitamin K₁ and 417 for the synthetic; those reported by Karrer were 318 and 324, respectively (un-

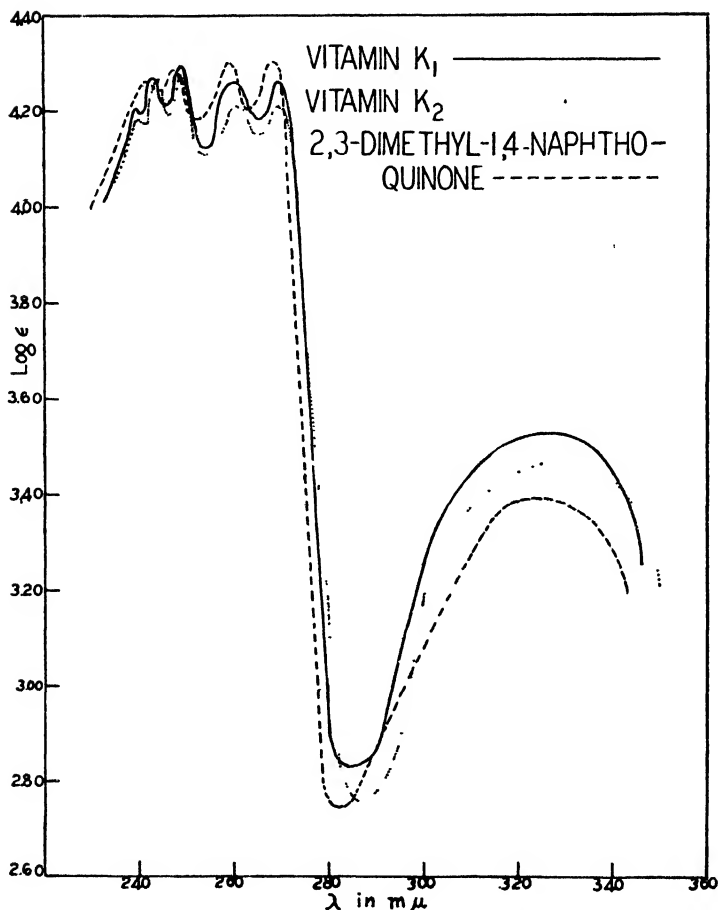


FIG 1. Ultraviolet absorption curves of vitamin K₁, vitamin K₂, and 2,3-dimethyl-1,4-naphthoquinone.

Later Binkley *et al.* (36) reported a value of 540 for the extinction coefficient of a single vitamin preparation. Since all subsequent determinations agree approximately with the value given in the first paper, the figure

published data). Ewing subsequently made additional determinations on specimens of the same preparations and obtained 432 and 439 for the natural and the synthetic product, respectively. In view of the agreement between the values for the extinction coefficient of vitamin K₂ reported by Karrer (119, 120) and by Ewing (71), the cause for the difference in the values for vitamin K₁ is not apparent.

Although the value of the extinction coefficient of our preparations differed from the value given by Karrer, Almquist's (16, 18) assays of specimens of vitamin K₁ supplied by the two research groups showed close agreement.

of 540 should not be considered as characteristic of pure vitamin K₁. After determining the extinction coefficients of a large number of samples of both natural and synthetic vitamin K₁, Dr. Ewing (unpublished data)

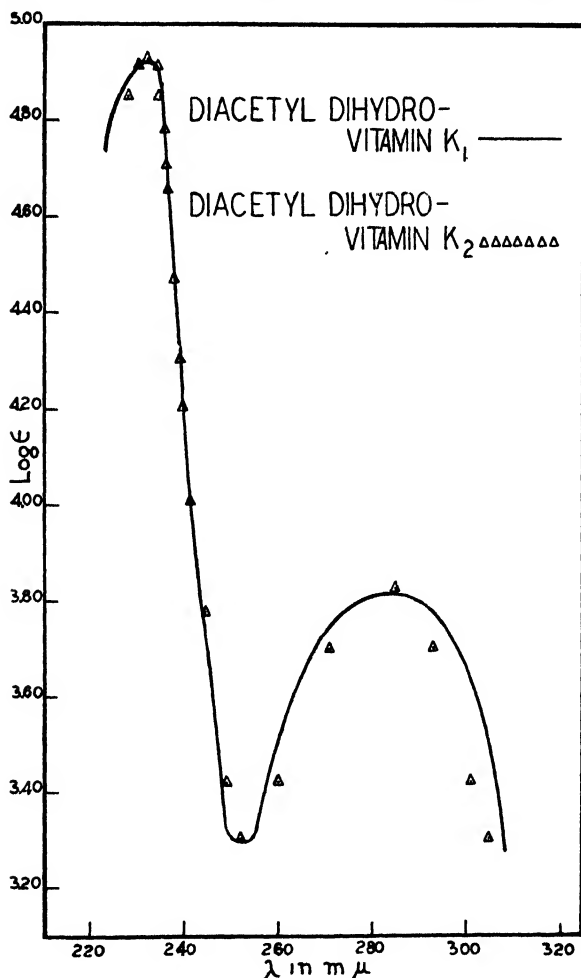


FIG. 2. Ultraviolet absorption curves of diacetate of dihydrovitamin K₁ and diacetate of dihydrovitamin K₂.

concluded that with hexane as solvent $E_{1\text{cm.}}^{1\%} = 430 + 10 (\log E_m = 4.29)$. This agrees with the most recent values reported by Tishler *et al.* (188), who state that the most reliable value for vitamin K₁ is $\log E_m = 4.26$ (alcohol). Since Ewing *et al.* (71) found that, by using hexane as a

solvent, slightly higher maxima were obtained and the fine structure was better defined than in ethyl alcohol, the values reported by the two groups are well within the experimental error (see table 1). Through the courtesy of Dr. Ewing, curves for vitamin K_1 , vitamin K_2 , and 2,3-dimethyl-1,4-naphthoquinone are reproduced (figure 1). It should be noted that a maximum which had previously escaped detection has been found at 240 $m\mu$.

Table 1 gives the molar extinction coefficients found for the most intense absorption band of vitamins K_1 and K_2 and of several simple 2,3-dialkyl-1,4-naphthoquinones. It is significant that the values for all of these compounds show such good agreement, especially when the difference due to the use of different solvents is taken into account. This observation is excellent evidence that $\log E_m = 4.27$ to 4.29 (hexane) for vitamins K_1 and K_2 is the correct value.

The ultraviolet absorption curves for the diacetates of dihydrovitamins K_1 and K_2 are reproduced in figure 2. The values for $\log E_m$ at 232.5 $m\mu$ for the two compounds show good agreement (4.93 for the diacetate of dihydrovitamin K_1 , and 4.93 for the diacetate of dihydrovitamin K_2). Fieser (78) reports a slightly higher value ($\log E_m = 4.98$) for the diacetate of vitamin K_1 , whereas Karrer *et al.* (120) find that $\log E_m = 4.93$.

B. OXIDATION-REDUCTION POTENTIAL

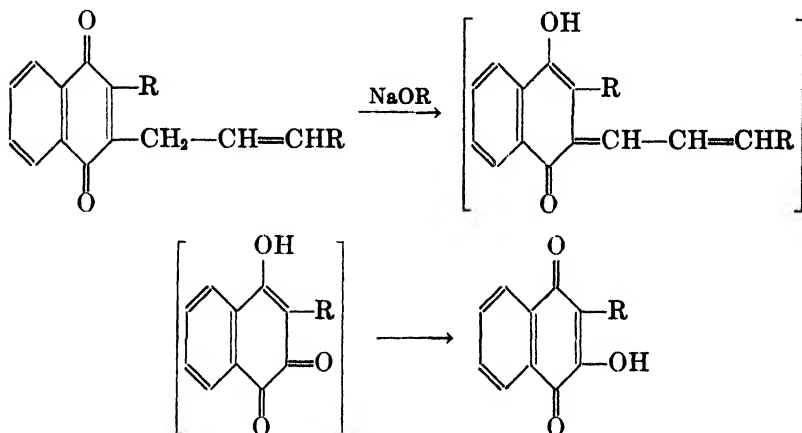
Karrer *et al.* (119) reported an oxidation-reduction potential $E_m = +0.005$ volt for the vitamin from alfalfa. Riegel (162, 164) found the potential E_0 for pure vitamin K_1 to be 363 millivolts at 20°C. According to Riegel, Karrer did not use the customary method for determining the oxidation-reduction potentials of quinones; consequently, a satisfactory comparison of the two values cannot be made. From a consideration of the pH of the solvent used, Riegel calculated Karrer's value of the oxidation-reduction potential E_0 to be about 400 millivolts.

C. COLOR REACTIONS

Dam *et al.* (57) observed that sodium ethylate reacts with vitamin K_1 to give a transient blue color which fades to reddish brown. Almquist (12), working with partially purified preparations, found that the amount of color developed agreed well with bioassays, whereas Fernholz (74) found that some potent preparations of the vitamin failed to give the reaction. All investigators now agree that the reaction is characteristic of 2-methyl-3-phytyl-1,4-naphthoquinone.

Fieser *et al.* (85) obtained the characteristic color with 1,4-naphthoquinones containing at least one allyl group in the quinonoid ring and isolated 2-hydroxy-3-allyl-1,4-naphthoquinone as the end-product of the

reaction of sodium ethylate with 2,3-diallyl-1,4-naphthoquinone. Later it was established that synthetic vitamin K₁ gave phthiocol as one product of the color reaction (77, 78). The following mechanism was proposed for this reaction (85):



Essentially the same interpretation of the first phase of this color reaction was advanced by Karrer (117).

The formation of phthiocol from vitamin K₁ by alkali led to the suggestion that the phthiocol isolated from the human tubercle bacilli may have arisen from the alkaline cleavage of a K-type vitamin.

Another color reaction which proved to be useful in determining the structure of vitamins K₁ and K₂ was Craven's color test (38, 48, 137). 1,4-Naphthoquinones substituted in the 2-position give a deep blue color with ammoniacal alcoholic ethyl cyanoacetate, while 2,3-disubstituted derivatives give no color.

IX. SIMPLE 1,4-NAPHTHOQUINONES AND RELATED COMPOUNDS

As soon as McKee *et al.* (144) announced that vitamins K₁ and K₂ are quinones and gave data which could be interpreted only in terms of the α -naphthoquinones, a number of investigators became interested in the potencies of the naphthoquinones. The first report was by Almquist (13), who apparently correlated the production of vitamin K potency by bacteria with Anderson's phthiocol from tubercle bacilli. Although this compound possesses potency, its activity is not comparable with that of the vitamin from alfalfa. A very important observation was made by Ansbacher (29, 31, 72), who found that 2-methyl-1,4-naphthoquinone is more active than vitamin K₁. Although other groups of investigators (14, 15, 181) failed in their early work to observe the great activity of this

compound, they now agree that it is at least twice as active as vitamin K₁ (16, 18, 67, 69, 161, 180, 183, 190).

A. QUINONES WITHOUT POTENCY

The following quinones, which do not belong to the 1,4-naphthoquinone series, have been prepared and tested for vitamin K activity: benzoquinone (15, 30, 181), toluquinone (30, 181), *p*-xyloquinone (141, 181), phlorone (30), diallyl-1,4-benzoquinone (82), 2-methoxy-3-methyl-1,4-benzoquinone (141), 2,3-dimethyl-1,4-benzoquinone (141), trimethylbenzoquinone (30, 141), triethylbenzoquinone (61), thymoquinone (61, 181), diamylhydroquinone (181), duroquinone (30, 61, 141), 2,3,5-trimethyl-6-phytyl-1,4-benzoquinone (90), trimethyl- γ -oxybutylbenzoquinone (61), diallyl-1,4-benzohydroquinone diacetate (82), α -tocopherylquinone (61, 133), anthraquinone (15, 61, 141), 1,2-dihydroxyanthraquinone (15), anthraquinone- β -sulfonic acid (181), 1,1,3-trimethyl-1,4-dihydroanthraquinone (92), 2,7-dinitrophenanthraquinone (141), 2-hydroxy-3-methylanthraquinone (141), quinalizarin (141), rufigallol (141), purpurin (141), 1,1-dimethyl-3-*tert*-butyl-1,4-dihydroanthraquinone (92), 2-(δ -methyl- γ -pentenyl)-1,4-dihydroanthraquinone (92), dihydroanthraquinone diacetate (181), and phenanthraquinone (61, 141, 181). Of these quinones only a few have been reported to possess antihemorrhagic activity. Ansbacher (30) found phlorone to be active at 1 mg. Kuhn *et al.* (133) reported that α -tocopherylquinone is active at 10 mg., but Dam *et al.* (61) found it to be inactive at a level of 0.0044 mg. per gram of body weight. Triethylbenzoquinone shows slight activity (61). Martin and Lischer (141) found that purpurin is active at 0.1 mg. and that rufigallol, anthragallol, and duroquinone are active at 10 mg.

A large number of naphthoquinones containing hydroxyl groups have been studied. These include phthiocol (13, 18, 29, 72, 79, 181), 3,4-dihydroxy-1,2-naphthoquinone (134), 2-allyl-3-hydroxy-1,4-naphthoquinone (134), 2-*n*-butyl-3-hydroxy-1,4-naphthoquinone (134), α -lapachone (80, 134), lapachol (14, 80, 82, 85, 133), lomatiol (14, 80, 82), hydrolapachol (15, 80, 82), phthiocol ethyl ether (15), phthiocol octadecyl ether (15), phthiocol phytyl ether (15), dihydropthiocol triacetate (15), phthiocol monoacetate (15, 18), hydroxyhydrolapachol (80, 82), lomatiol methyl ether (82), lapachol methyl ether (82), juglone (133), 3,5,6,7,8-pentaoxy-2-allyl-1,4-naphthoquinone (133), 2- α -heptenyl-3-hydroxy-1,4-naphthoquinone (83), 2-*n*-heptyl-3-hydroxy-1,4-naphthoquinone (83), and β,β -dimethyldihydrofurano-1,4-naphthoquinone (134). As compared with vitamin K₁ or 2-methyl-1,4-naphthoquinone, these compounds are relatively inactive. Phthiocol, which has been reported to be from $\frac{1}{200}$ to less than $\frac{1}{1000}$ as active as the vitamin, has special significance, since it

was the first simple 1,4-naphthoquinone reported to have vitamin K activity.

TABLE 2
2-Substituted 1,4-naphthoquinones and derivatives

COMPOUND AND REFERENCE	APPROXIMATE WEIGHT OF ONE UNIT
	γ
Naphthalene (15)	—
1,2-Naphthoquinone (73, 181)	—
1,4-Naphthoquinone (15, 61, 73, 133, 181)	1000
2-Methyl-1,4-naphthoquinone (XIV) (18, 29, 61, 67, 72, 134, 170, 180, 181, 183, 190)	0.5
2-Ethyl-1,4-naphthoquinone (73, 170, 181)	100
2- <i>n</i> -Propyl-1,4-naphthoquinone (73, 85)	1000
2-Allyl-1,4-naphthoquinone (73, 82, 83, 181)	>1000
2- <i>n</i> -Hexadecyl-1,4-naphthoquinone (73)	>1000
2- <i>n</i> -Octadecyl-1,4-naphthoquinone (73)	>1000
2-Geranyl-1,4-naphthoquinone (89, 90)	—
2-Farnesyl-1,4-naphthoquinone (89, 90)	—
2-Phytyl-1,4-naphthoquinone (61, 89, 90, 121)	50
1,4-Naphthohydroquinone diacetate (181)	2000
2-Oxymethyl-1,4-naphthoquinone acetate (61)	Weak
2-Methyl-1,4-naphthohydroquinone diacetate (29, 61, 72, 181)	1
2-Phytyl-1,4-naphthoquinone oxide (91)	—
2-Farnesyl-1,4-naphthoquinone oxide (91)	500

Owing to the differences in assay procedures used in various laboratories and our utilization of data published by other investigators, the values given in these tables for the potencies of these compounds are only approximations. However, there is little doubt that the order of magnitude of the potencies is correct.

Many different units are in use, but in these tables the values given are in terms of our unit. This unit is the specific antihemorrhagic activity of 0.8 mg. of a standard alfalfa extract. On the basis of this unit and by the procedure of assay used in this laboratory, the potency of 2-methyl-1,4-naphthoquinone is 2000 units per milligram and that of vitamin K₁ is 1000 units per milligram.

As an example, the following method was used in assigning potencies to compounds which have not been assayed in this laboratory: Dam gives the activity of vitamin K₁ as 12,000,000 and that of 2-methyl-1,4-naphthoquinone monoxime as 5,000,000 of his units. The weight of one of our units of the oxime is therefore 2.4 γ ; the approximate figure 2 γ is given in the table. In these tables, — in the column giving the weight of the unit indicates that the compound was inactive for the amount used in the assay.

The following naphthoquinones which are substituted in the benzenoid ring failed to show appreciable vitamin K activity: 2,6-dimethyl-1,4-naphthoquinone (73, 83), 2,7-dimethyl-1,4-naphthoquinone (83), 2,3,5-trimethyl-1,4-naphthoquinone (61), 2,3,6-trimethyl-1,4-naphthoquinone

(61), 3,5,7-trimethyl-1,4-naphthoquinone (61), 3,6,7-trimethyl-1,4-naphthoquinone (61), 3,5,7-trimethyl-1,2-naphthoquinone (61), and 2,6-dimethyl-3-phytyl-1,4-naphthoquinone (78).

Table 2, which includes the 2-substituted 1,4-naphthoquinones and derivatives, shows that, if the alkyl group is increased beyond the methyl group, the potency is lost and is not regained even when the alkyl group contains sixteen to twenty carbon atoms.

TABLE 3
2,3-Disubstituted 1,4-naphthoquinones and derivatives

COMPOUND AND REFERENCE	APPROXIMATE WEIGHT OF ONE UNIT
	γ
2-Methyl-1,4-naphthoquinone (XIV) (18, 29, 61, 67, 72, 134, 170, 180, 181, 183, 190)	0.5
2,3-Dimethyl-1,4-naphthoquinone (82, 85, 133)	50
2-Methyl-3-bromo-1,4-naphthoquinone (181)	10,000
2-Methyl-3-amino-1,4-naphthoquinone (18)	75
2-Methyl-3-benzyl-1,4-naphthoquinone (78, 87)	>100
2-Methyl-3-trimethylallyl-1,4-naphthoquinone (87)	>100
2-Methyl-3-cinnamyl-1,4-naphthoquinone (78, 87)	100
2-Methyl-3-geranyl-1,4-naphthoquinone (78)	25
2-Methyl-3-farnesyl-1,4-naphthoquinone (89)	10
2-Methyl-3-phytyl-1,4-naphthoquinone (18, 33, 61, 69, 72, 76, 137, 144, 183, 185)	1
2-Methyl-3-palmityl-1,4-naphthoquinone (18)	4
2-Methyl-3-n-octadecyl-1,4-naphthoquinone (73)	1,000
2-Ethyl-3-phytyl-1,4-naphthoquinone (78)	>160
2,3-Diallyl-1,4-naphthoquinone (73, 82)	>1,000
2-Methyl-1,4-naphthoquinone dimer (18)	20
2,3-Dibromo-2-methyl-1,4-dioxotetrahydronaphthalene (181)	10,000
2,3-Dimethyl-1,4-naphthoquinone oxide (91)	25
2-Methyl-3-cinnamyl-1,4-naphthoquinone oxide (91)	Weak
Disodium 2,3-dimethyl-1,4-naphthohydroquinone disulfate (88)	500

B. POTENT 1,4-NAPHTHOQUINONES

When 2-methyl-1,4-naphthoquinone (table 3) is substituted in the 3-position by groups other than an alkyl group, e.g., bromine, amino, etc., the most potent derivative is only $\frac{1}{100}$ as active as the parent compound. 2,3-Dimethyl-1,4-naphthoquinone is about $\frac{1}{10}$ as active as the compound not substituted in the 3-position. From his study of a series of 2,3-dialkyl-1,4-naphthoquinones, Fieser (78) reported a certain specificity in structure associated with antihemorrhagic activity. With the exception of 2,3-dimethyl-1,4-naphthoquinone, activity began to appear as the alkyl

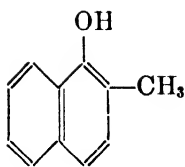
groups were increased in size beyond six to eight carbon atoms, became appreciable in the 2-methyl-3-cinnamyl (ten carbons) and 2-methyl-3-geranyl (eleven carbons) compounds, and reached a peak in vitamin K₁ (twenty-one carbons) and vitamin K₂ (thirty-one carbons). Fernholz *et al.* (73) pointed out that the inactivity of 2-*n*-octadecyl, 2-methyl-3-*n*-octadecyl, and similar long-chain substituted 1,4-naphthoquinones does not fit into this picture. However, there does appear to be an increase in activity with increase in the length of the chain in the 3-position, so long as this chain is β,γ -unsaturated and is made up of isoprenoid units. As pointed out in the discussion of table 2 and as illustrated by the contrasting inactivity of 2-ethyl-3-phytyl-1,4-naphthoquinone, the methyl group in the 2-position is highly specific. Since on a weight basis 2-methyl-1,4-naphthoquinone is twice as active as vitamin K₁, whereas, on a molar basis, the two are of about equal activity, Fieser postulated that 2-methyl-1,4-naphthoquinone merely served as a component for the synthesis of vitamin K₁ in the organism. Almquist and Klose (18) pointed out that the ratio of the activities of vitamins K₁ and K₂ is directly proportional to their respective contents of 2-methyl-1,4-naphthoquinone, and that the potencies of these vitamins are equivalent to about 80 per cent of the 2-methyl-1,4-naphthoquinone contained in them. These investigators propose that these relationships can be best explained by assuming that the long side chains of vitamins K₁ and K₂ are split off to the same extent.

C. OTHER POTENT COMPOUNDS

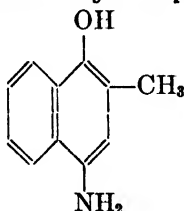
Tishler and Fieser and coworkers have prepared a number of methyl-naphthols (XI) and methyltetralones (XVI) (table 4). Of these, the compounds which on oxidation could give 2-methyl-1,4-naphthoquinone have potencies of the same order as vitamin K₁. β -Methylnaphthalene is active at 1 mg., but 1-amino-2-methylnaphthalene is considerably more active. In a study of the following series of compounds^a—(1) β -methylnaphthalene, (2) 1-nitro-2-methylnaphthalene, (3) 1-amino-2-methylnaphthalene, (4) 2-methyl-1-naphthol (XI), (5) 4-amino-2-methyl-1-naphthol (XII), (6) 2-methyl-1,4-naphthohydroquinone (XIII), and (7) 2-methyl-1,4-naphthoquinone (XIV)—compounds 1, 2, and 3 showed low potencies, but compounds 5, 6, and 7 showed appreciably more activity. 2-Methyl-1-naphthol was about $\frac{1}{10}$ as active as 2-methyl-1,4-naphthoquinone.

Fieser made the significant observation that the oxides of 1,4-naphthoquinones possess the same order of potency as the compounds from which

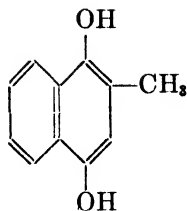
^a Richert, Binkley, Thayer, and Doisy (unpublished data and also references 80 and 187).

Antihemorrhagic compounds

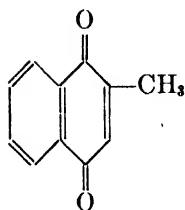
XI
2-Methyl-1-naphthol



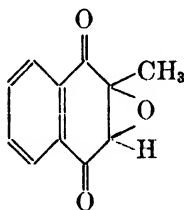
XII
4-Amino-2-methyl-1-naphthol



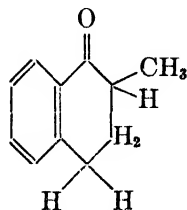
XIII
2-Methyl-1,4-naphthohydroquinone



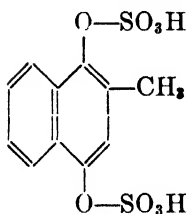
XIV
2-Methyl-1,4-naphthoquinone



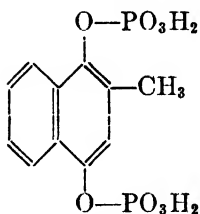
XV
2-Methyl-1,4-naphthoquinone oxide



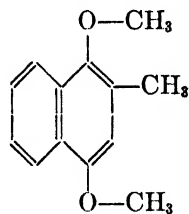
XVI
2-Methyl-1-tetralone



XVII
2-Methyl-1,4-naphthohydroquinone
disulfuric acid ester



XVIII
2-Methyl-1,4-naphthohydroquinone
diphosphoric acid ester



XIX
1,4-Dimethoxy-2-methylnaphthalene

they are derived (tables 4 and 5). These compounds are colorless, are easily reduced to quinones, and do not give the Dam-Karrer color test. On this basis, he postulated that vitamin K₀ (33) of Ansbacher might be the oxide of vitamin K₁.

The hydroquinones are equal in activity to the corresponding quinones, whereas the diacetates are one-half as active. This statement holds for the hydroquinones and the diacetates of all 1,4-naphthoquinones that have been assayed (18, 31, 72, 181). However, Dam *et al.* (61) report considerably less activity for the diacetate of dihydrovitamin K₁.

TABLE 4

Naphthols, tetralones, esters of 2-methyl-1,4-naphthoquinone, and related compounds

COMPOUND AND REFERENCE	APPROXIMATE WEIGHT OF ONE UNIT
	γ
2-Methyl-1-naphthol (XI) (187, 189)	1
3-Methyl-1-naphthol (187, 189)	1
1-Methyl-2-naphthol (187)	—
3-Methyl-2-naphthol (187, 189)	—
4-Methyl-1-naphthol (187, 189)	—
2-Methyl-1-naphthylamine (187, 189)	5
β -Methylnaphthalene (187)	1000
3-Methyl-1-tetralone (187, 189)	1
2-Methyl-1-tetralone (XVI) (187, 189)	1
2-Methyl-5,6,7,8-tetrahydro-1,4-naphthoquinone (75)	1000
2-Methyl-1,4-naphthoquinone oxide (XV) (79, 91)	5
2-Methyl-1,4-dimethoxynaphthalene (XIX) (31)	5
2-Methyl-1,4-naphthoquinone monoxime (61)	2
2-Methyl-1,4-naphthohydroquinone diacetate (18, 31)	1
2-Methyl-1,4-naphthohydroquinone dipropionate (31)	1
2-Methyl-1,4-naphthohydroquinone dibenzoate (31)	1
2-Methyl-1,4-naphthohydroquinone di- <i>n</i> -butyrate (31)	1.25
2-Methyl-1,4-naphthohydroquinone diisobutyrate (31)	5
2-Methyl-1,4-naphthohydroquinone di- <i>n</i> -valerate (31)	1.25
2-Methyl-1,4-naphthohydroquinone diisovalerate (31)	3
2-Methyl-1,4-naphthohydroquinone dimesitoate (187, 189)	200
2-Methyl-1,4-naphthalenedioxydiacetic acid (32)	2000

TABLE 5

Vitamin K₁ and derivatives

COMPOUND AND REFERENCE	APPROXIMATE WEIGHT OF ONE UNIT
	γ
Vitamin K ₁ (18, 33, 61, 69, 72, 76, 137, 144, 183, 185)	1
Dihydrovitamin K ₁ diacetate (36, 137)	2
β , γ -Dihydrovitamin K ₁ (90, 189)	6
2-Methyl-3-phytyl-5,8-dihydro-1,4-naphthoquinone (90)	5
β , γ , 5,6,7,8-Hexahydrovitamin K ₁ (75, 90, 189)	>2
Vitamin K ₁ oxide (91)	1
Dipotassium vitamin K ₁ hydroquinone disulfate (88)	>500
Dihydrovitamin K ₁ diphosphoric acid (88)	25
Naphthotocopherol (75, 90)	300
	(>1000)
2-Methyl-3-isophytyl-1,4-naphthoquinone (33)	15
2-Methyl-2-phytyl-2,3-dihydro-1,4-naphthoquinone (188)	50

The high potency of 2-methyl-1,4-dimethoxynaphthalene (XIX) (table 4) is interesting. Owing to the difficulty of biological hydrolysis of methyl ethers of phenols,⁶ Ansbacher believed it possible that this compound acted as a whole. Likewise, he favored this explanation for the lower activity of the complex esters of 2-methyl-1,4-naphthohydroquinone rather than differences in the rate of hydrolysis or in the rate of absorption. As Tishler *et al.* (187) noted, another possibility to be considered is direct oxidation of the derivative to 2-methyl-1,4-naphthoquinone. However, these investigators consider the low activity of the dimesitoyl derivative of 2-methyl-1,4-naphthoquinone ($\frac{1}{250}$ as active as the dibenzoate) to be an indication that hydrolysis plays an important part. Since a biochemical study on the mode of action of these compounds has not been reported, any statement on this question is premature.

Table 5 is a compilation of the derivatives of vitamin K₁ and compounds which may be considered to be related in structure. Like the effect on the potency of 2-methyl-1,4-naphthoquinone, complete reduction of the benzenoid ring destroys the activity, while other reduction products show diminished activity. Fieser *et al.* (90) reported naphthotocopherol to be active at 300 γ , whereas Fernholz *et al.* (75) found it to be inactive at 1 mg., while the oxidation product was active at 300 γ . The disulfate and the diphosphate of dihydrovitamin K₁ show considerably less activity than vitamin K₁.

D. POTENT WATER-SOLUBLE COMPOUNDS

Since the 1,4-naphthoquinones and the natural vitamins are oil-soluble and must be used in conjunction with bile salts in oral therapy, and since a large proportion of patients in need of therapy cannot be treated orally because of nausea, intestinal obstruction, or other complications, it was important to find a compound of high activity which could be dissolved in an aqueous medium for intravenous use.⁷ Although 2-methyl-1,4-naphthoquinone and 2-methyl-1,4-naphthohydroquinone have been used successfully, the solubility in saline is too low for their use in a convenient volume. The compounds listed in table 6 possess the same order of potency as 2-methyl-1,4-naphthoquinone and are water-soluble by reason

⁶ Although it is commonly believed that the methyl ethers of phenols are not easily hydrolyzed, Westerfeld (201) and Stroud (178) have shown that certain ethers of this type are hydrolyzed by monkeys and by rabbits, respectively.

⁷ The water-soluble compounds can be used orally as well as intravenously. Warner and Flynn (198) have shown that the potassium salt of the disulfuric acid ester of 2-methyl-1,4-naphthohydroquinone is readily absorbed from the intestinal tract of rats without the aid of bile salts. Smith and Owen (171) have found that patients respond to the oral administration of 1 mg. of 4-amino-2-methyl-1-naphthol without the addition of bile salts.

of a free amino group which forms a water-soluble hydrochloride, or an acidic group which forms a salt, or glucose residues which increase the water solubility. On a molecular basis, sodium 1,4-naphthohydroquinone disulfuric acid ester is one-third as active as the quinone itself. Foster *et al.* (95, 134) and Almquist (18) claim that tetrasodium 1,4-naphthohydroquinonediphosphoric acid ester (XVIII) is about 1.5 times as active as 2-methyl-1,4-naphthoquinone on a molecular basis and conclude that the vitamin probably acts through this derivative in the animal body. On the other hand, Ansbacher (32) reports that it is less active than the parent quinone or hydroquinone.

TABLE 6
Highly potent water-soluble compounds

COMPOUND AND REFERENCE	APPROXIMATE WEIGHT OF ONE UNIT
	γ
2-Methyl-1,4-naphthoquinone (XIV) (18, 29, 61, 67, 72, 134, 170, 180, 181, 183, 190)	0.5
2-Methyl-1,4-naphthohydroquinone (XIII) (18, 31, 67, 170).	0.5
2-Methyl-1,4-naphthohydroquinone monosuccinate (161)	0.7
2-Methyl-1,4-naphthohydroquinone disuccinate (61)	1
4-Amino-2-methyl-1-naphthol hydrochloride (XII) (18, 61, 67, 161).	1
4-Amino-3-methyl-1-naphthol hydrochloride (161)	1
Disodium 2-methyl-1,4-naphthohydroquinone disulfate (XVII) (32, 79, 88, 161)	6
Tetrasodium 1,4-naphthohydroquinone diphosphoric acid ester (XVIII) (18, 32, 94, 95, 134)	0.6-5
2-Methyl-1,4-naphthohydroquinone diglucoside (162).	?
2-Methyl-1,4-naphthoquinone sodium bisulfite complex (162)..	?

Since the methods of synthesis of the quinones mentioned in the previous paragraphs and tables are mainly standard procedures which had been described prior to the development of the vitamin K field, a complete review of these methods will not be given. The methods of synthesis of vitamin K₁ have general application for the introduction of other substituted allylic groups into the 3-position in 2-methyl-1,4-naphthohydroquinone and into the 2-position in 1,4-naphthohydroquinone (89). The method of Barbot (34) has been used by Fernholz *et al.* (73) and by Karrer *et al.* (118) for the production of β -substituted tetralins. The ketones were reduced by the Clemmensen method, and the resulting hydrocarbons were dehydrogenated with sulfur to give the desired alkylnaphthalenes. The quinone was made by chromic acid oxidation. Karrer *et al.* (121) prepared 2-phytylnaphthalene by dehydration of the tertiary alcohol

obtained by the reaction between β -naphthylethylmagnesium bromide and 2,6,10-trimethylpentadecanone-14. After the preparation of the dibromide, the compound was oxidized to a 1,4-naphthoquinone and debrominated with zinc. In a second method naphthylacetylene was condensed with the ketone and the triple bond was hydrogenated to give the same tertiary alcohol. Fieser prepared the oxido derivatives by treatment of the quinone with hydrogen peroxide (189). Ring-closure methods were used for the preparation of the tetralones (189). 4-Amino-2-methyl-1-naphthol was prepared by the reduction of the monoxime of 2-methyl-1,4-naphthoquinone. The esters, ethers, and hydrogenation products were obtained by the usual methods. The references given after each compound refer to its preparation and bioassay.

X. CLINICAL WORK WITH ANTIHEMORRHAGIC COMPOUNDS

A. PHYSIOLOGICAL CONSIDERATIONS

Before passing to a brief discussion of the clinical use of vitamin K, it seems advisable to review certain physiological observations upon which the later clinical work was based. Since Wedelius (199) reported the first case of fatal choleric bleeding in 1683, many investigations have been directed at the problem of the hemorrhagic tendency in obstructive jaundice. The various components of the clotting system were examined for abnormalities, but it was not until 1935 that Quick, Stanley-Brown, and Bancroft (158) devised a satisfactory method for the determination of prothrombin and showed that in obstructive jaundice the prothrombin concentration may be markedly reduced. In the same year, Hawkins and Whipple (103) found a hemorrhagic condition in dogs 3 to 4 months after an operation which established a complete biliary fistula. Besides the spontaneous bleeding, they noted a prolonged clotting time which they thought was due to prothrombin deficiency. Continuing the work, Hawkins and Brinkhous (102) in 1936 showed that the delayed clotting is due to a deficiency of prothrombin.

At about the same time (1936), Greaves and Schmidt (100) found spontaneous bleeding and decreased coagulability in rats with bile fistulas. Oral administration of bile corrected the hemorrhagic condition. Later (1937) they (98, 101) showed that the condition of the rats having bile fistulas could be cured by vitamin K and bile, and that bile was important in the absorption of vitamin K.

In the meantime, the study of vitamin K was progressing and Schønhøyder (167), one of Dam's associates, showed in 1936 that the hemorrhagic syndrome of chicks is due to a deficiency of prothrombin. In 1937 Greaves and Schmidt (101) showed that administration of vitamin K relieved the prothrombin deficiency in rats having bile fistulas and stated that, although

bile alone sufficed, it was effective because of its capacity to promote the absorption of vitamin K.

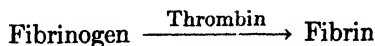
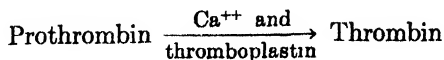
At this time (1937) Quick (153) correlated the different observations in a short note and suggested that vitamin K should prove useful in the hemorrhagic condition which frequently accompanies obstructive jaundice. In this clinical condition there is a deficiency of prothrombin, and in experimental animals such a deficiency can be corrected by the administration of the vitamin K and bile. Though Quick did not immediately test his hypothesis on patients, it was only a few months later that reports on the therapeutic use of vitamin K appeared almost simultaneously from three different groups of investigators: Warner, Brinkhous, and Smith (197) in January, 1938; Butt, Snell, and Osterberg (45) on February 2, 1938; Dam and Glavind (60) on March 26, 1938.

B. DETERMINATION OF PROTHROMBIN

Since it has been found that the clotting time may be within normal limits when the concentration of prothrombin has been reduced to one-third of the normal value, it is obvious that the determination of prothrombin constitutes a more delicate index than gross clotting time of the danger of impaired clotting. In obstructive jaundice the clotting time may be normal when the prothrombin value indicates a close approach to the hemorrhagic condition.

A discussion of the methods of determining prothrombin would take us too far afield; consequently the reader will be given only a brief statement on this point. Almost simultaneously, Quick, Stanley-Brown, and Bancroft (158) and Warner, Brinkhous, and Smith (196) published methods for the quantitative determination of prothrombin. These methods and modifications of them (2, 97, 108, 110, 114, 122, 124, 125, 151, 154, 175, 202) have been used extensively in establishing the desirability of vitamin K therapy in patients and in the control of the efficacy of the treatment.

The methods are based on the time required for blood or plasma to pass from a fluid to a gel state under optimal conditions. This time bears an established relationship to the concentration of prothrombin. The gel is produced by the action of thrombin, an enzyme (?) which is formed from prothrombin, on fibrinogen. The fibrinogen is converted to the insoluble fibrin.



C. TREATMENT OF OBSTRUCTIVE JAUNDICE

Following Quick's suggestion as to the use of vitamin K in obstructive jaundice and the appearance of the three papers previously mentioned, several groups of workers added information on the therapeutic value of vitamin K. The importance of antihemorrhagic compounds in obstructive jaundice seems to be thoroughly established (1, 24, 25, 42, 96, 109, 113, 115, 128, 130, 148, 152, 159, 160, 166, 172, 173, 176, 177, 179, 192). It should be pointed out that impairment of absorption in the intestine or severe damage to liver function (26, 39, 43, 44, 172, 200) may prevent a response to the administration of vitamin K; in the former case the difficulty can be overcome by the injection of the synthetic water-soluble antihemorrhagic compounds.

In an experiment by Zuckerman *et al.* (203), conducted on a patient with a total biliary fistula, slight bleeding from gums occurred after 2 weeks on a low fat, vitamin-K-free, and bile-free diet, and a week later bleeding from the tongue and vagina. A vitamin K concentrate was fed for 4 days without an effect on bleeding; feeding of the patient's own bile for 5 days was without effect, but when the bile and vitamin K were administered together the prothrombin and clotting times approached normal values and bleeding ceased.

In addition to impaired absorption of vitamin K, owing to the absence of bile from the intestine, certain intestinal conditions,—e.g., obstruction and severe diarrheal diseases, such as ulcerative colitis, sprue, and celiac disease,—may cause hypoprothrombinemia (47, 70, 112, 116). Intravenous therapy with one of the simple water-soluble antihemorrhagic compounds should be effective.

Although deficiency in vitamin K of dietary origin in the human should be rare on account of the widespread distribution of antihemorrhagic compounds, Kark and Lozner (114) have reported four cases of mild deficiency. The prothrombin values were slightly low before treatment, but after the administration of a vitamin K concentrate without added bile the values were restored to normal.

D. HEMORRHAGIC DISEASE OF THE NEWBORN

Another important therapeutic use of vitamin K merits consideration. In 1937, Brinkhous, Smith, and Warner (41) found a low prothrombin value in the hemorrhagic disease of the newborn. This was confirmed by Waddell and his collaborators (193, 194), who reported two newborns with prothrombin times in excess of 6 min. Within 2 hr. after the administration of vitamin K, the values had fallen to less than 1 min. Since from 25 to 40 per cent of the mortality of the newborn is due to the hemorrhagic syndrome, Waddell undertook the study of the effect of adminis-

tration of vitamin K to the mother before labor. This work has been extended by Hellman and Shettles (105), who have reported a comparison of values of the newborn with the values of the mothers and have shown that values of the newborn can be increased by medication of the prospective mother with vitamin K. A number of additional contributions (40, 104, 106, 123, 129, 131, 132, 140, 147, 150, 155, 156, 157, 165, 169, 191, 195) have been made to the study of the hemorrhagic disease of the newborn.

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The attention of the reader is directed to the following publications, which cover certain aspects of vitamin K more adequately than this review:

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THE SOLUBILITY OF GASES IN LIQUIDS

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Received January 10, 1941

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I. INTRODUCTION

The solubility of gases in liquids was one of the physical properties studied by the early chemists. Many well-known names appear in the literature, as Henry in 1803, Berthelot, Bunsen, Carius, and Roscoe in 1855, Winkler in 1889, and other later workers. In general, the solubility of gases in liquids has been studied by investigators who have wished to have these particular bits of data in the investigation of the gas or, more infrequently, of the liquid or the solution. Few were interested in the phenomenon itself and in studying general behavior in the solubility of gases in liquids. The literature cited shows the names of a large number of workers,—men who studied the solubility of all gases in a wide variety of liquids, men who used all types of apparatus and experimental conditions, and men who exercised various degrees of experimental technique in producing their data. The results vary from those of high precision to those little more than qualitative. Many investigators neglected some of the important factors in solubility or failed to record all the data. Most of the work has been done at random pressures near atmospheric, and the values corrected to 760 mm. by means of Henry's law; this is usually permissible. Many workers have failed to indicate whether the pressure was total or partial, thus introducing a large uncertainty. The data usually are calculated to either the Bunsen or the Ostwald coefficient, and if the worker does not state which is used, the values diverge as the temperature increases from 0°C.

Some attempts have been made to correlate gas solubility with the properties of the liquid or the solution. Most of these are entirely empirical and are based on a few data secured by one investigator.

It is the purpose of this paper to review the work done on the solubility of gases in liquids and to discuss the various factors of importance in this field of work. Solubilities at high pressures have not been included, because of the special apparatus and technique used. The merits and limitations of the various experimental methods are described. The equations proposed for correlating gas solubility with other variables have been collected and rewritten in a uniform system. All existing data on the solubility of gases in liquids have been tabulated, so that the user may know the range of temperature and pressure of the experiments and the relative precision of the data. It has not been possible in this review to collate the data, but an indication is given of the probable precision of the results on the basis of method used, the completeness of the data, and the consistency of the results among themselves.

II. METHODS AND APPARATUS

¹ Many methods of procedure and kinds of apparatus have been used in the measurement of gas solubility. Most of these can be classed definitely

as either chemical or physical. Chemical methods depend on specific chemical properties of the gas, and thus can be used with only a limited number of gases. Physical methods usually depend on no such properties and are thus more general. When suitable chemical methods are available, however, they are frequently more accurate and usually much quicker.

A. PHYSICAL METHODS

The physical methods used are quite varied. Most of them are saturation methods, in which the measurement is that of the quantity of the gas necessary to saturate a quantity of initially gas-free solvent. Some are extraction methods, in which the measurement is that of the volume of gas that can be extracted from a quantity of saturated solution.

1. Removal of gases from solvent

In saturation methods, the liquid must be gas-free at the start. In extraction methods, the gas is all to be extracted. Hence, in either case, the complete removal of gas from a liquid is important. This is not an easy matter. In saturation methods the problem is not as difficult as in extraction methods, since loss of gas is permissible, loss of solvent usually is, and usually the solvent as obtained contains only atmospheric gases. The presence of traces of the atmospheric gases probably has little effect on the solubility of others, though there is no authority in the literature on this point. Buchanan (36) made quantitative measurements of the extraction of carbon dioxide from water and from aqueous salt solutions by boiling. He distilled solutions saturated with the gas, and tested portions of the distillate for carbon dioxide with barium hydroxide. He found that when the solvent was distilled water, the first eighth of the distillate contained nearly all the gas, the second eighth a trace, and the rest none. When the solution contained sulfates, he found it necessary to boil nearly to dryness to remove all of the carbon dioxide. If the sulfate were removed by the addition of barium chloride, the resulting solution gave up its gas about as readily as did distilled water. Leduc (176) found that even after boiling distilled water a long time, it gave up gas bubbles on freezing. Successive freezing in a vacuum did not free the water completely from gas. Metschl (207) made measurements of the gas liberated when a solution saturated at several atmospheres pressure was shaken at 1 atmosphere. Water and organic liquids were the solvents, and the gases included hydrogen, oxygen, nitrogen, and carbon dioxide. All of these except the latter were readily liberated, in amounts predicted by the solubility figures in the literature; hence equilibrium was evidently reached. In the case of carbon dioxide, however, the results indicated either that gas

was lost before measurement or that equilibrium was not reached by shaking. Porter (246) made statements about the difficulty of removing gas from water, but evidently these were due largely to misunderstanding of the solubility curves (see Sillitto (286)). Seyler (281) raised the temperature of water samples containing oxygen, to see if the oxygen were lost. He concluded that, when the solution was not shaken, the gas stayed in solution in going 5 or 6 degrees above the equilibrium temperature, but that shaking established equilibrium.

The usual method of preparing gas-free liquid for solubility measurements has been boiling, followed by cooling in a vacuum. Bunsen (37, 38, 39, 40) used this method, and most others have followed him. Hibben (120) has applied vacuum sublimation to prepare gas-free liquid.

Paunov (234) found that, under the influence of ultrasonic frequencies, the amounts of gas absorbed decrease by about 50 per cent.

2. Saturation methods

The general principle most frequently employed is the measurement of a volume of gas before it is brought in contact with a quantity of gas-free solvent, and its measurement again after equilibrium is established. The volume dissolved is found by difference. There are many different arrangements of the essential parts of the apparatus to achieve this end. Henry (118) used this method in 1803. The impure gases that he used and the limitations in material necessarily caused results of a very low degree of accuracy. Bunsen (37, 38, 39, 40) used an apparatus that he designed, which employed the same principle. His apparatus is shown in figure 1.

The calibrated absorption tube *e* is fastened at the bottom to a small iron band *b*; this screws into the small iron stand *a*. By this arrangement the open end of the tube can be screwed tight against a plate of rubber covering the lower surface of the stand. Thus the tube can be completely sealed. On each side of the stand are two steel springs *c*, which fit into two upright grooves in the wooden base, *f*, of the apparatus. When the tube and stand are in place, it is easy to open or close the absorption tube by giving it a turn to the right or left. The water jacket *g* is held firmly in place by the screws *ii*. The tube *r* is for the purpose of pouring in mercury and removing it, so that any desired pressure in the absorption bulb can be obtained by adjusting the mercury level in the water jacket. The temperature of the water can be read on the thermometer *d*. The water jacket is closed on top by a hinged lid. The piece of rubber *s* serves to hold the tube in place during the shaking necessary in the process of absorption.

The experiment is conducted in the following manner: A volume of the

gas to be examined is first collected in the tube over mercury, and its volume, temperature, and pressure are read. A measured volume of air-free water is introduced under the mercury into the tube, which is then sealed by being screwed tightly against the rubber plate. The tube is

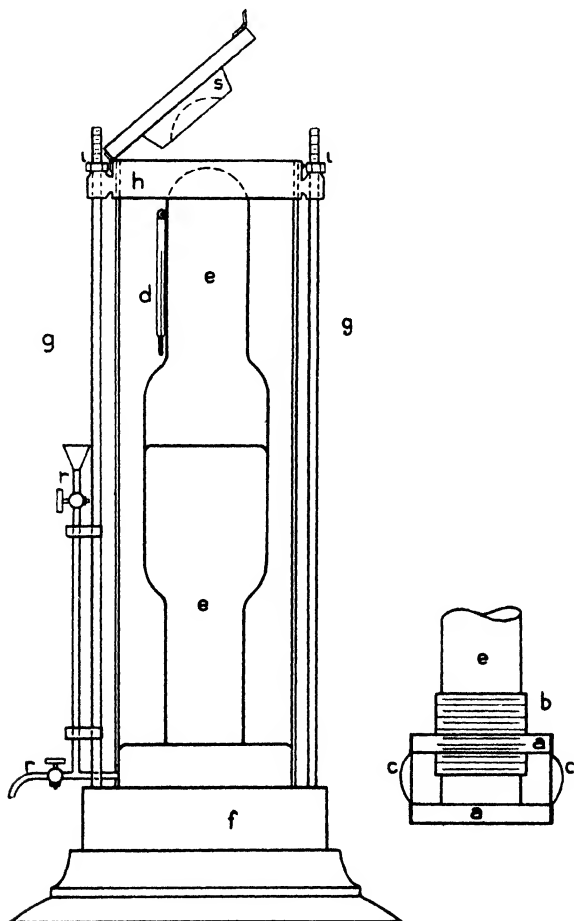


FIG. 1. Bunsen's apparatus for the determination of the solubility of gases

then placed in the water jacket, which contains some mercury at the bottom. The pressures within and without are equalized by turning the tube slightly. The tube is then sealed again by turning, and vigorously shaken. This agitation, with opening and closing of the tube, is repeated many times, until no further change of volume is perceptible. The ob-

servations necessary for the measurement and reduction of the residual gas are then made.

Bunsen applied this method with considerable success to the measurement of the solubilities of the common gases, including hydrogen, oxygen, nitrogen, air, methane, carbon dioxide, ethylene, and ethane, in water. Carius (44) used Bunsen's apparatus for the same gases in alcohol; Schickendantz (267) used it for ethane in water; and Than (306) used it for propylene in water. Khanikoff and Luginin (149) used an apparatus similar to Bunsen's for the carbon dioxide-water system at pressures up to several atmospheres. Maclaurin (192) modified Bunsen's method to avoid contact of the solution with mercury, for the measurement of the solubility of oxygen in potassium cyanide solutions. Ramsay and coworkers (250, 251) used Bunsen's method to determine the solubility of argon and of helium in water. Tower (314) used it with nitric oxide in sulfuric acid. Sander (266) used a modification of Bunsen's method for the measurement of the solubility of carbon dioxide in water and in organic solvents at high pressures, up to 140 atmospheres. For the measurement of the solubility of a number of gases in cyclohexanol Cauquil (46) used a method which, from his description, appears to be similar to Bunsen's.

Ostwald (231a) introduced a method which proved to be much better than Bunsen's and within a few years almost entirely displaced it. The fundamental difference was that the gas is measured in a buret connected to the absorption vessel, rather than in the absorption vessel itself. The buret and leveling tube are similar to those used in the Hempel gas analysis equipment. From one arm of a three-way stopcock a flexible capillary tube connects to an absorption bulb, similar to a gas-sampling bulb with three-way cocks at the ends. Ostwald usually used lead for the flexible capillary, though silver and platinum are mentioned. This assembly of equipment is capable of almost infinite variation and refinement. McDaniel (204) used the apparatus in substantially its original form; his apparatus is shown in figure 2.

The essential parts are a gas buret, A, connected by a capillary tube, M, to an absorption pipet B, so that the entire apparatus is of glass. The buret and pipet are inclosed within water jackets, the temperature of each being regulated by electrically heated coils in the water. The whole apparatus is clamped solidly on a rigid frame, so that it can be taken in the hands and shaken to bring the gas into intimate contact with the liquid.

In operation, the pipet B is filled completely with gas-free solvent. The source of gas is connected to the apparatus at T, and the gas is passed through a saturator H filled with the solvent. First, the stopcocks C and D are turned so that the capillary is filled with the gas, C is then closed and D is opened to the buret A, which is filled with the gas; measurement

is made by adjusting the mercury leveling bulb F. The source of gas then is disconnected, D and C are adjusted to allow gas to flow into pipet B, stopcock G is opened, and a measured volume of solvent is withdrawn to give a gas volume in the pipet B. The mercury level in the buret is adjusted, stopcocks C and D are closed, and the entire apparatus is shaken to dissolve gas in the solvent. At intervals, additional gas from the buret

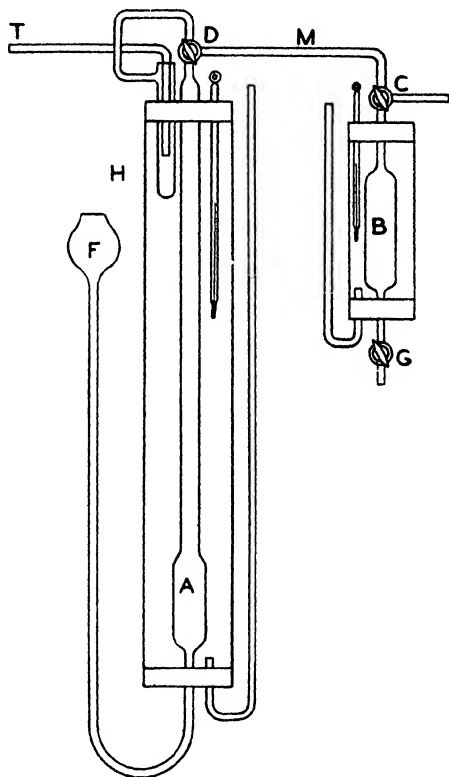


FIG. 2. Ostwald type of apparatus for the determination of the solubility of gases

is added to the pipet to maintain the pressure as the gas dissolves. The original volume of the liquid in the pipet minus that withdrawn gives the volume of solvent in which gas is absorbed. To the volume of gas remaining in the buret at equilibrium is added the volume of liquid withdrawn which is the new vapor space above the solution. From the volume of gas absorbed and the volume of solvent used, the solubility of the gas is calculated.

While Bunsen's apparatus was ordinarily used in its original form,

Ostwald's seems rarely to have been. The chief variations have been the provision for agitation of the liquid and provision for a gas-liquid interface. Usually the gas buret has been stationary and the absorption flask has been shaken. This procedure necessitates a flexible joint between the two. McDaniel shook the whole apparatus (see figure 2) as a unit. Stern (296) used a glass-capillary spiral to provide the flexible joint. Maxted and Moon (202), using an apparatus designed from that of Just (147), also used a glass spiral. In order to get more freedom from the joint, Steiner (295) used a platinum capillary, as did Timofeev (312). Scenenov (279) and Estreicher (77) also used metal spirals. Curry and Hazleton (61) used a copper capillary.

If an iron bob inclosed in glass is placed in the absorption vessel and moved by a magnet the connection between this vessel and the buret can be rigid. Åkerlöf (3), Antropoff (5), Cady, Elsey, and Berger (41), Cassuto (45), and Wright and Maass (344) have made use of this idea. Showalter and Ferguson (284) used the ground-glass joint of a stopcock to provide the flexible joint.

Lunge (186) fastened the buret and absorption vessel together with a short stub of rubber tubing, thus providing a flexible joint. Others have used the same idea. Lannung (172) assembled both parts in one rigid piece and shook the whole assembly to agitate the liquid. His apparatus is shown in figure 3. Absorption bulb A is made to contain various volumes in different pieces of apparatus. The gas buret and manometer are combined in B C, behind which is a measuring scale read with a telescope. The buret is calibrated from mark *a* to mark *b*. The entire apparatus is attached to an aluminum frame so that it can be shaken. The apparatus is evacuated at *s* and mercury admitted at 1 until the apparatus is filled as far as *s*, the manometer tube C and the movable reservoir *g*. To the ground joint *s* is attached an L-tube, the other end of which dips into the pure solvent; the solvent is drawn into A by letting mercury run out at 1 until A is about half full. The L-tube is detached, and the solvent is de-aerated by suction at *s* and confined in such a way as not to be in contact with stopcocks. The entire apparatus is placed in an air thermostat and allowed to come to equilibrium. Buret B is filled with the gas through stopcock 4 and the volume measured when saturated with solvent vapor. The solvent surface is lowered from *f* by letting mercury out at 1. The entire apparatus is agitated until equilibrium is established, the volume in B is measured, and the solubility is calculated from the decrease in volume.

In McDaniel's apparatus, the absorption vessel was filled with liquid at the start, and, to provide a suitable gas-liquid interface, liquid was drained from the bottom. The amount so drained was weighed, and thus the corresponding volume of gas that replaced it was found. Steiner

(295), Timofeev (312), and Secenov (279) provided the gas-liquid interface in the same way. As an alternative, mercury may be placed in the bottom of the vessel, to be drained out and weighed in the same manner. Cady, Elsey, and Berger (41) and Lannung (172) used this idea. Lunge

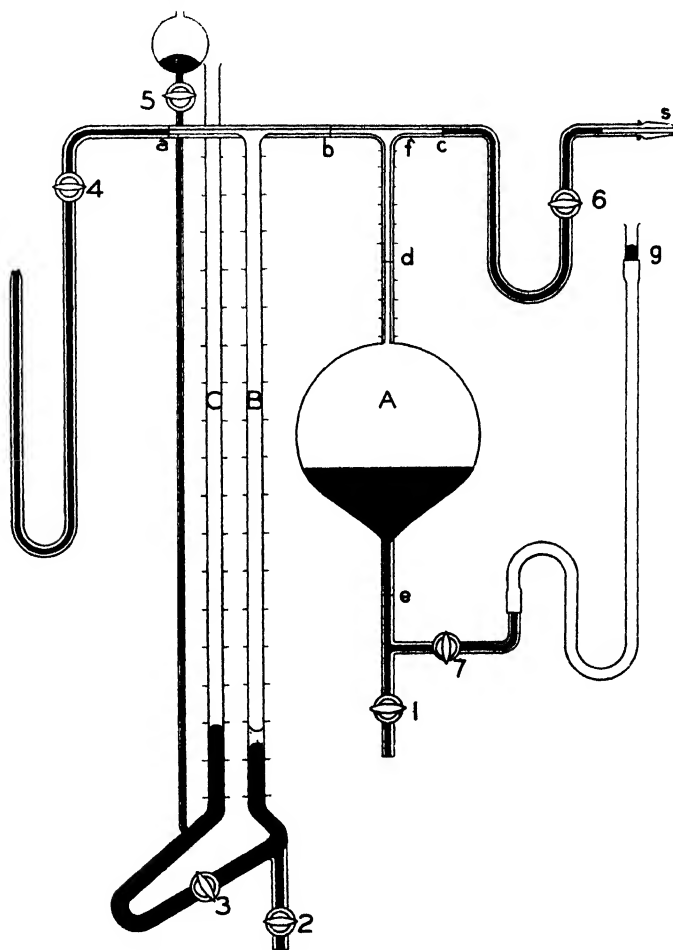


FIG. 3. Ostwald type of apparatus, as used by Lannung

(186) connected the bottom of the vessel to a leveling bulb of mercury, which could be lowered to let mercury out of the vessel. After equilibrium was reached, the bulb could be raised, filling the vessel again, and no correction was necessary for gas that replaced the liquid drained. Christoff (49) brought the liquid and gas in contact before measuring the gas

volume. He found that the rate of solution was such that no appreciable volume of gas was dissolved during the measurement. Estreicher (77) and Drucker and Moles (71) left a vacuum above the liquid when the vessel was filled, which volume was filled with gas from the buret after the gas was measured. Others have done the same. Manchot (197) used an absorption vessel with two compartments, one filled initially with gas, and the other, a smaller one placed above it, with liquid. After measurement of the gas volume, the liquid could be drained into the lower compartment, filling it only part full and leaving considerable gas above it. The upper compartment was filled with gas from the lower one during the draining. Markham and Kobe (201) used a similar apparatus, in which the liquid-gas interface could be made larger relative to the volume of solvent. Usher (320) filled the absorption vessel with gas before measurement, and let in a known amount of liquid later. In his measurements with solutions, he put the solid solute in the absorption vessel before filling it with gas, then introduced the gas and made the buret reading, and later introduced a known amount of solvent, effecting solution of the solid solute in the vessel itself. Such a procedure involved the assumption that the solid did not absorb the gas. Homfray (128), working on *p*-azoxyphenetole, put the solid crystals in contact with the gas, then observed the change in the volume of the gas when the crystals were melted and later heated to the anisotropic state.

Some investigators have saturated the gas with liquid vapor before filling the buret, while others have kept the gas in the buret dry. Horiuchi (133) has discussed the relative merits of both methods. If the gas in the buret is saturated, the vapor pressure of the solvent is of little consequence. If the gas is dry, however, the vapor pressure must be known accurately, since all gas coming into the free space above the liquid in the absorption vessel picks up vapor, increasing its volume to an extent determined by the vapor pressure. On the other hand, if the gas in the buret is saturated, any part of the apparatus that is not in the thermostat may collect condensed solvent if the thermostat is above room temperature. The capillary between the buret and the absorption vessel is usually out of the thermostat. Drops of liquid in this capillary would make the pressure adjustment in the buret uncertain. If the gas in the buret is dry, the temperature of the whole apparatus can be changed and thus a range of temperature can be covered with one filling.

As ordinarily used, Ostwald's apparatus has involved at least one mercury surface in contact with the gas, and sometimes in contact with the solvent as well. This feature is a serious handicap when dealing with systems that react with mercury. To avoid this difficulty, Wright and Maass (344), working with hydrogen sulfide, used a modification in which

the volume of gas remained constant, while the pressure varied and was measured with a manometer having a glass diaphragm. Bancroft and Belden (10) used a similar arrangement.

Cady, Elsey, and Berger (41) stated that the violent shaking frequently used to effect equilibrium could cause pressures at the surfaces of the absorption vessel far in excess of that measured by the manometer. Thus the solvent would be supersaturated with respect to the pressure read on the manometer, and the solubility results would be too high. To test this point, Morgan and Pyne (216) used an apparatus in which the gas was bubbled through the liquid repeatedly. They felt that no such supersaturation as Cady, Elsey, and Berger mention could result in their apparatus, and that, if they could check the solubility values found by others who had used the shaking method, the question of this supersaturation would be answered. The values obtained by Morgan and Pyne for the system carbon dioxide–water checked exactly the values found by the shaking method. Thus, in many instances at least, this method has caused no appreciable error. Hainsworth and Titus (107) also used a method in which the gas was bubbled repeatedly through the liquid. They approached equilibrium from both sides, getting the same values in each case. Thus they were certain that equilibrium had been established. Bancroft and Belden (10) found that 30 sec. of shaking established equilibrium in the hydrogen sulfide–aniline system, and that identical values were found when equilibrium was approached from either side. Rakestraw and Emmel (249) found that when sea water was shaken with air and the solution was allowed to stand till bubbles were no longer visible, the nitrogen content was 2 per cent higher than the equilibrium value.

In most methods of determining gas solubility, the average solubility throughout a volume of liquid is determined. The solubility may change with depth as a result of the hydrostatic head. Few experimenters have considered this point. Morgan and Richardson (218) determined the effect of hydrostatic head on the solubility of oxygen in water and found it to have the same effect as any other pressure.

The change in the volume of the solution as a gas dissolves necessarily introduces a certain error. The error so introduced is probably less than other experimental errors in the case of the gases of small solubility. Markham and Kobe (201) showed that the solubility of carbon dioxide in aqueous solutions might be in error up to 0.1 per cent for this reason.

Instead of measuring the gas volumetrically, gravimetric means may be used. The gas-free solution is weighed, then saturated with gas by bubbling, and then weighed again, to give the solubility. The solvent carried away by the escaping gas can be caught and weighed, and correction

applied therefor. This method is limited to the more soluble gases. It has been used by Raoult (254) on the ammonia-water system, including aqueous salt solutions; by Prytz (247) on hydrogen sulfide and carbon dioxide in water; by Naumann (223) on cyanogen in water; by Baskerville and Cohen (11) on phosgene in organic solvents; and by others.

3. *Extraction methods*

These methods involve the extraction by some means of the gas contained in a quantity of saturated solution, and the measurement of the volume of gas so extracted. Thus the procedure of the saturation method is reversed. As mentioned earlier, the difficulty of complete extraction is of special interest here.

These methods are, in general, useful for the analysis of naturally occurring solutions, such as sea water. They were first used in this way. Later they were applied by many to artificially saturated solutions.

Reichardt (255) described an apparatus for boiling the gas out of water and collecting it for measurement. Tornøe (313) also described such an apparatus. Numerous others,—Dittmar (65), Hamberg (111), Petterson and Sonden (242), Clowes (53), Winkler (341), Weigert (331), James (142), and Ruppin (264),—have described apparatus designed for the same purpose. Buchanan (36) boiled aqueous carbon dioxide solutions, catching the distillate in barium hydroxide for analysis. The same idea has been applied to other systems (see the work of Calingaert and Huggins (42) on ammonia and water).

Other investigators have extracted the gas from solution by evacuation; e.g., Bohr (25) pumped carbon dioxide from its solution in water. The apparatus of Van Slyke (324, 241a) is the best known for this type of measurement (figure 4). It is used principally to determine the amount of gases dissolved in blood and blood fluids. The short pipet A contains 50 cc. and has several graduations on it; *a* corresponds to 2 cc. The pipet is connected to the manometer and to the mercury leveling bulb. The sample of gas solution is introduced through stopcock b by a special pipet in such a way that the solution does not come in contact with the air. Then by lowering the leveling bulb the gas solution is evacuated, and the pipet is shaken for 2 or 3 min. to assist in liberating the gas. The liberated gas is compressed into the volume *a* and the pressure read on the manometer. An empirical correction is made for the gases redissolved during the compression. The gases collected can be analyzed for carbon dioxide or oxygen by introducing the appropriate absorbent solution through stopcock b and determining the pressure after the particular component has been removed. Objections can be raised to this method because of the question concerning the extraction of dissolved gases and the corrections

applied to the results. However, the data presented by the authors indicate that satisfactory results were obtained. The method of saturating blood was adopted from Austin, Cullen, and Hastings (8). Conant and Scott (55), Kubie (170), Hawkins and Shilling (114, 115) and others have used the Van Slyke method. Orcutt and coworkers (230), in using it, applied a correction for the gas not extracted. Results have been ob-

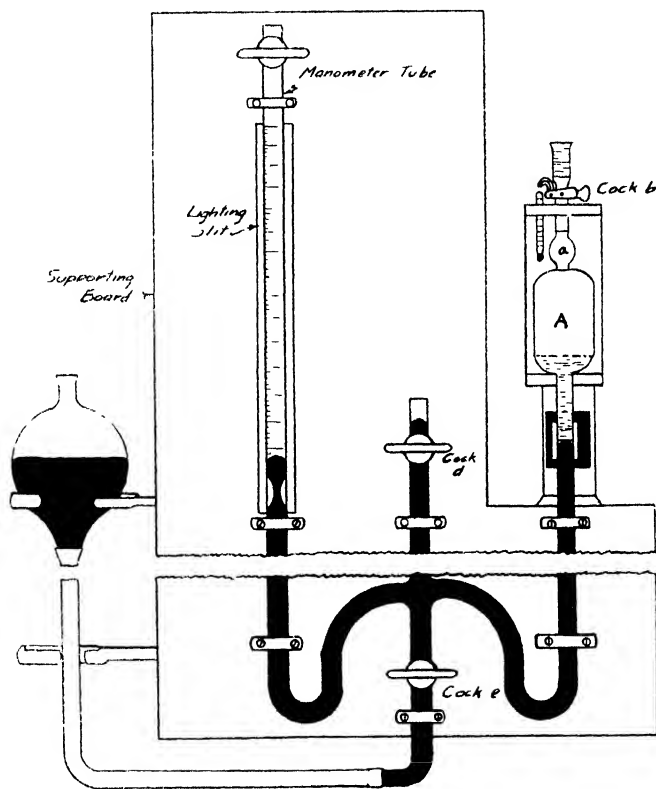


FIG. 4. Apparatus of Van Slyke

tained by this method that check very well those obtained by saturation methods. Scotti-Foglieni (277) described an apparatus for the saturation of liquid with gas, to be followed by analysis.

4. Miscellaneous methods

The foregoing methods have been used far more than other physical methods. A few others have been used to a slight extent.

The solubility of the radioactive gases has been measured by electrical means, determining the concentration in the liquid phase and in an inert gas phase, usually air. The concentration of the gas under investigation is necessarily very small. Trautenberg (315), Hofmann (127), Kofler (160, 161) and others have applied this method to radium emanation, Klaus (155) and Boyle (28) have applied it to thorium emanation, and Hevesy (119) has applied it to actinium emanation.

The solubility of a gas has sometimes been determined by the freezing-point lowering produced by the addition of the gas to saturation. This method is necessarily limited to one temperature for each solvent, and is uncertain because of the possibility of association or dissociation of the gas in the liquid phase. It is further limited to those systems which give a freezing-point lowering of sufficient magnitude. Prytz (247) applied this method to hydrogen sulfide and carbon dioxide in water. Garelli and Falciola (97) applied it to carbon dioxide, nitrous oxide, acetylene, and hydrogen sulfide in water and in several organic liquids. Their results checked values found in other ways. Garelli (96) and Garelli and Monath (98) also used this method. Falciola (79) reported that, if oxygen, methane, or several other gases were present, the freezing point might be raised by the addition of the above gases.

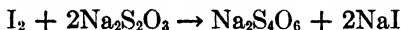
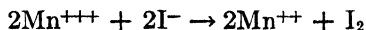
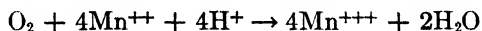
Schwab and Berninger (275) used a special method for solubility measurements, based on the change in pressure in a bubble rising in a column of liquid. The results are not very accurate.

B. CHEMICAL METHODS

In the case of the less soluble gases, the solution has usually been saturated by bubbling the gas through at atmospheric pressure, after which the content of gas has been determined by suitable means. The total pressure was that of the atmosphere, and the solvent was assumed to have the same vapor pressure as in the pure state. Obviously, the very soluble gases like ammonia and the hydrogen halides affect the vapor pressure of the solvent and must be treated differently. Their vapor pressures have usually been measured by bubbling an inert gas through the solution, and absorbing the gas under investigation from the gas stream in some medium where its quantity can be determined. Very low vapor pressures can be found in this way. The introduction of inert gas probably affects the solubility of the very soluble gas but little.

Schutzenberger (274), Mohr (213); Winkler (336), and Levy and Marboutin (178) developed chemical methods for the determination of oxygen in solution. That of Winkler, involving the oxidation by the oxygen of

manganous hydroxide, the reduction by iodide of the manganic hydroxide so produced, and the titration of the liberated iodine, has proved to be



much the best. This method has been of value both in the analysis of natural waters and in solubility measurements. König and Mutschler (164), Tiemann and Preusse (311), Roscoe and Lunt (261), Clowes and Biggs (54), and Naylor (224) report the use of these methods. Coste and Andrews (59) showed that Winkler's method is not accurate in the presence of ammonium salts in quantity.

The more soluble gases are usually acid or alkaline in nature, and frequently have an oxidizing or reducing character as well. Thus several methods for analysis are usually available.

The oxidizing nature of chlorine has usually been the basis for its analysis. Roscoe (259), however, precipitated it as silver chloride.

Lewis and Keyes (179) determined hydrogen cyanide by precipitating it as the silver salt.

The alkaline character of ammonia and of the amines has been the basis for their analysis. Doijer (66), Perman (239, 240, 241), Locke and Forssall (183) and others have used this property in their vapor pressure or solubility measurements. Hydrogen chloride is naturally analyzed as an acid (see Shunke (273) and numerous others). Carbon dioxide has been determined in solution as an acid by Bohr (26) and by Kosakevich (168). Phosgene was determined by Atkinson and coworkers (7) by treatment with alkali in excess, and back-titration. Kremann and Honel (169) determined acetylene by absorption in silver nitrate solution, followed by titration of the acid liberated.

Sulfur dioxide has usually been determined by its oxidation with iodine, as was done by Fox (92). This is also true of hydrogen sulfide (Goldschmidt and Larson (102)). Briner and Perrottet (31) found the solubility of ozone in water by shaking the water with air containing ozone, and later analyzing each phase for ozone, using potassium iodide and thiosulfate.

When inert gas is used to sweep the soluble gas from solution, in order to measure its partial pressure, there is some choice of the inert gas. Air was used by Doijer (66) and by Lofman (184). Gahl (95) used the gas produced by electrolysis of water for this purpose. The ease of regulating the flow of gas and the accuracy of its measurement have led to the frequent use of this method. Dolezalek (67), McLauchlan (205), and Gaus

(99) found the partial pressures of hydrogen chloride, hydrogen sulfide, and ammonia, respectively, using electrolytic gas. Stegmüller (294) used nitrogen for the same purpose in determining the partial pressure of hydrogen iodide.

Frequent measurements have been made of the distribution of a volatile substance between two liquids. If the solubility of the gas in one of the liquids is known, the distribution data provide means of finding approximately its solubility in the other liquid. Bell and Feild (16) determined the distribution ratio of ammonia between water and chloroform, and Smith (290) worked with amines distributed between xylene and water; other systems have been investigated.

III. SOLUBILITY RELATIONSHIPS

As may be expected in any field where many investigators have published, there exists a variety of ways in which these results have been expressed. Some are in common usage; others, which are seldom used, should be the ones in common usage. The investigators also have attempted to find some correlation between the properties of the liquid and the properties of the gas that will make it possible to predict the solubility of a gas in a given liquid from known properties or from a few solubility data.

A. METHODS OF EXPRESSING SOLUBILITY

1. *Bunsen absorption coefficient, α*

This coefficient was proposed by Bunsen (39) and was defined by him as follows: "The volume of gas, reduced to 0° and 760 mm. pressure of mercury, which is absorbed by the unit volume of liquid under the pressure of 760 mm. is called the absorption-coefficient, or coefficient of absorption." Although not so stated in the definition, his calculations show that the pressure meant is always the partial pressure of the gas. Bunsen used the ideal gas laws to reduce the gas volume to standard conditions. As these laws are not exact, the coefficients found by different methods, i.e., physical and chemical, can be expected to differ. Thus Markham and Kobe (201) showed that in the case of carbon dioxide at 0°C., deviations of 0.7 per cent were to be expected between the two methods. This deviation will increase as the behavior of the gas departs from the ideal gas laws.

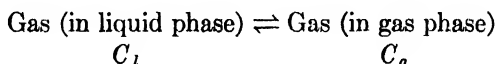
Many of the past workers have not controlled the total pressure carefully, so that the partial pressure of the gas has remained at 760 mm. Frequently the total pressure has been maintained at 760 mm. and the vapor pressure of the solvent has been neglected. Other workers have not used a partial gas pressure of 760 mm. but have corrected their actual results to this pressure by the use of Henry's law.

If the solubility is calculated according to the Bunsen coefficient, except that the amount of solvent is 1 g., the result is known as the Kuenen coefficient. For solutions this has been extended by Markham and Kobe (201) to mean the volume of gas (in cubic centimeters) at a partial pressure of 760 mm., reduced to standard conditions, dissolved by the quantity of solution containing 1 g. of solvent; thus it is proportional to gas molality. It is designated by S .

If the solubility is calculated as grams of gas dissolved per 100 cc. of solvent at the temperature of the experiment and a partial gas pressure of 760 mm., the result is known as the Raoult absorption coefficient.

2. Ostwald coefficient of solubility, L

This coefficient was defined by Ostwald (231a) as "the ratio of the volume of the absorbed gas to that of the absorbing liquid. If these are V_l and V_g , respectively, the solubility is $L = V_l/V_g$." For the reaction



the Ostwald coefficient of solubility can be written as

$$L = \frac{C_l}{C_g} = \frac{V_l}{V_g} \qquad (1)$$

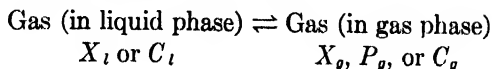
which represents the ratio of the concentrations of gas in the liquid phase and in the gaseous phase. This is in reality an equilibrium constant, and the Ostwald coefficient is independent of the *partial* pressure of the gas as long as ideality may be assumed. However, the temperature and total pressure must be designated to fix the value of the coefficient. If the total pressure is maintained at 760 mm., the volume of gas absorbed, reduced to 0°C. and 760 mm. by the ideal gas laws, per unit volume of liquid is frequently designated as β , an absorption coefficient.

As pointed out in discussing the Bunsen coefficient, early workers frequently did not distinguish between total pressure and partial gas pressure, or did not consider the vapor pressure of the solvent. Thus in many cases results reported as α really are β . Likewise, the results found by physical and by chemical methods differ by the departure of the gas from the ideal gas laws.

3. Henry's law constant

Henry (118) stated his law as "... under equal circumstances of temperature water takes up the same volume of condensed gas as of gas under

ordinary pressure." The modern presentation of this law for the ideal phases of a gas in equilibrium with a liquid is:



$$X_g = K_x X_i \quad (2)$$

$$P_g = K_1 X_i \quad (3)$$

For a dilute solution of the gas:

$$P_g = K_2 C_i \quad (4)$$

$$C_g = K_c C_i \quad (5)$$

Thus it is seen that the Ostwald coefficient L , equation 1 is the reciprocal of K_c .

The Henry law constant, K_2 , is a satisfactory though unwieldy method of expressing gas solubility and is the method used in the *International Critical Tables* (138a). It is noted that, the larger the value of K_1 , the lower is the solubility.

Henry's law has been used by many investigators to calculate their data from an experimental pressure to a partial gas pressure of 760 mm. Over the short range usually encountered, no error is introduced. However, the worker must keep in mind that the equations given are for ideal dilute solutions and should apply any necessary corrections.

4. Interconversion of expressions for the solubility

(a) From the Bunsen coefficient:

$$\beta = \alpha \frac{760 - P_i}{760} \quad (6)$$

As β is the solubility coefficient measured at a total pressure of 760 mm., α is decreased from its partial pressure of 760 mm. by applying Henry's law.

$$L = \alpha \frac{T}{273} = \beta \frac{T}{273} \frac{760}{760 - P_i} \quad (7)$$

The Ostwald coefficient is calculated from α by correcting the gas volume to the temperature at which absorption was carried out.

$$S = \frac{\alpha}{\rho(1 - u)} \quad (8)$$

If the solubility is expressed per gram of solvent in a solution, the factor ρ ($1 - u$) gives the grams of solvent per cubic centimeter of solution.

$$K_1 = \frac{17.033 \times 10^6 \rho}{\alpha M_s} + 760 \quad (9)$$

The units of K_1 are those of pressure, expressed as millimeters of mercury. Only for the very soluble gases does the constant term of 760 mm. alter appreciably the significant figures of the value for K_1 calculated in the first term of the equation, and thus it usually is neglected.

$$K_2 = \frac{17033}{\alpha} \quad (10)$$

The units of K_2 are (mm. Hg) (liters of solvent)/mole of gas (see equation 4).

(b) From the Ostwald coefficient, L :

$$\alpha = L \frac{273}{T} \quad (11)$$

$$\beta = L \frac{273}{T} \frac{760 - P_s}{760} \quad (12)$$

$$K_c = 1/L \quad (13)$$

(c) From the Henry law constant, K_1 :

$$\alpha = \frac{17.033 \times 10^6 \rho}{(K_1 - 760)M_s} \quad (14)$$

The 760 mm. in the denominator may be neglected unless it is appreciable in comparison with K_1 , that is, unless the number of moles of gas dissolved appreciably affects the total moles of solution.

5. Nomenclature

It is desirable at this point to tabulate the nomenclature recommended for the expression of gas solubilities. The equations used here have been rewritten to conform as closely as possible to this system.

A = work done in dissolving one mole of gas;

C_g = concentration, as gram-moles per liter, of gas in the gas phase;

C_l = concentration, as gram-moles per liter, of gas in the liquid phase;

C_s = concentration, as gram-moles per liter, of salt in the liquid phase;

K_1 = Henry's law constant (to fit equation 3);

L = Ostwald coefficient (defined on page 536);

M_g = molecular weight of gas;
 M_s = molecular weight of solvent;
 P_g = partial pressure of gas;
 P_s = partial pressure of solvent;
 P_t = total pressure;
 R = gas constant;
 S = unit gas solubility (defined on page 536);
 T = temperature, degrees Kelvin;
 T_c = critical temperature of gas, degrees Kelvin;
 V_l = volume of the gas in the liquid phase;
 V_s = volume of the solvent;
 X_g = mole fraction of gas in the gas phase;
 X_l = mole fraction of gas in the liquid phase.

a, b, c, k, n = arbitrary constants;
 d = differential operator;
 e = base of natural logarithms;
 \ln = natural logarithm;
 \log = common logarithm;
 m = molality of salt;
 t = temperature, degrees Centigrade;
 u = decimal fraction of solute in solution.

α = Bunsen coefficient (defined on page 535);
 β = solubility coefficient (defined on page 536);
 γ = activity coefficient of the dissolved gas;
 μ = ionic strength of the salt;
 ρ = density of the solution.

Where necessary for distinguishing between the property of a solution and that of the pure solvent, the property of the solvent has been given the zero subscript.

B. VARIATION OF SOLUBILITY WITH PRESSURE

Henry (118) was the first to show the variation of gas solubility with pressure. With crude apparatus and impure gases, he performed experiments the results of which he summarized as follows: "The results of at least fifty experiments, on carbonic acid, sulfuretted hydrogen gas, nitrous oxide, oxygenous and azotic gases, with the above apparatus, establish the following general law: that, under equal circumstances of temperature, water takes up, in all cases, the same volume of condensed gas as of gas under ordinary pressure." Bunsen (39) used his method to confirm Henry's conclusions, using the carbon dioxide-water system. After this

confirmation of Henry's law by Bunsen, others performed experiments on other systems to test it. His law is accepted as the normal behavior of gas-liquid systems to such an extent that frequently only the deviations from it are noted.

The less soluble gases have been found to satisfy the law well at moderate pressures. Thus Morgan and Richardson (218) found that at 25°C. the system oxygen-water satisfied Henry's law in the pressure range 175 to 760 mm. Kireev and Romanchuk (151) found that hydrogen and methane in xylene, in ethylene chloride, and in several petroleum fractions satisfied Henry's law in the temperature range -20°C. to +40°C. at pressures from 50 to 760 mm. Briner and Perrottet (31) experimented with ozone in water, finding Henry's law to hold. The gas phase was air containing 0.3 to 9 per cent ozone. Boyle (29) confirmed Henry's law for solutions of radon in water and in several other solvents. The concentrations were necessarily very low, and air was present. Findlay and Shen (86) found hydrogen in water to satisfy Henry's law at 25°C. over the pressure range 750 to 1400 mm.

More data are available for gases of intermediate solubility, such as carbon dioxide. Roscoe (258, 259) tested Henry's law in the case of the chlorine-water system, in which it apparently failed; however, he varied the pressure by the addition of inert gas. Perman (238) worked with several gases in water, using an extraction method which gave only the relative pressures. He found that hydrogen sulfide, carbon dioxide, and chlorine satisfied Henry's law. Findlay and coworkers (81, 82, 83, 84, 85, 86, 87) tested Henry's law as applied to carbon dioxide and nitrous oxide in water, and to carbon dioxide in aqueous solutions of alcohol and several electrolytes, at 25°C. The pressure range was 250 to 1400 mm. The results show that the law is satisfied exactly in this range. Buch (35) tested Henry's law for the carbon dioxide-water system from pressures of 1 atmosphere to 1/20,000 of an atmosphere, and found it to hold. Khanikoff and Luginin (149) worked with carbon dioxide and water to test Henry's law. At the temperature they used (15°C.) the law failed, as they stated, at 4 atmospheres pressure. Actually, deviations of 5 per cent appeared in their data at less than 2 atmospheres. Vukolov (328, 329) tested the law as applied to the solubility of carbon dioxide in chloroform and carbon disulfide. He found maximum deviations of 4.5 per cent in the pressure range 36 to 760 mm. Secenov (279) experimented with carbon dioxide and aqueous salt solutions. He concluded that at low pressures (about one-third of an atmosphere) all salt solutions follow Henry's law. Few data were presented to justify this conclusion. Stern (296) found the solubility of carbon dioxide in several organic solvents at -78° and -59°C. over the pressure range 50 to 760 mm. Using Bunsen's coefficient, he found that Henry's law was not satisfied. However, at the temperatures

used the perfect gas law did not hold. When the results were expressed as Ostwald coefficients, the law held. Lewis and Keyes (179) found that the pressure of hydrogen cyanide over its aqueous solution was proportional to the molarity. Bancroft and Belden (10), working with hydrogen sulfide in aniline at 22°C. and at pressures up to 1200 mm., found Henry's law to hold.

The very soluble gases usually have not satisfied Henry's law, except at elevated temperatures or at very low concentrations. Sims (287) tested Henry's law for two systems and found that in both cases it failed at lower temperatures, but that, as the temperature increased, the deviations became less until at 50°C. the sulfur dioxide-water system satisfied the law, and at 100°C. the ammonia-water system did also. Smith and Parkhurst (291) measured the solubility of sulfur dioxide in water and in solutions of calcium and magnesium bisulfites, and found that Henry's law was satisfied at pressures up to 800 mm. in the temperature range 5° to 60°C. Quite contradictory conclusions have been reached for the ammonia-water system. Gaus (99) found that ammonia in water and in salt solutions at 25°C. satisfied Henry's law up to about 1 normal concentration with respect to ammonia (14 mm. pressure). Abegg and Reisenfeld (1) reached the same conclusions. Roscoe and Dittmar (260) found that Henry's law failed when applied to the systems ammonia-water and hydrogen chloride-water. Calingaert and Huggins (42) found that at 100°C. the ammonia-water system deviated from Henry's law even at low concentrations. They concluded that the deviations could be explained by the electrolytic dissociation of the ammonia in solution (*cf.* MacDougall (190a)). Klarmann (154), however, verified Henry's law for the same system at concentrations of 0.5 to 1/128 normal, at 0°C. Perman (238), who obtained only relative pressures, found that ammonia, hydrogen chloride, and sulfur dioxide in water did not satisfy Henry's law at room temperatures. Doijer (66) tested Henry's law for the ammonia-water system at 60°C., in the pressure range 8 to 60 mm., and found it to hold.

In cases where the gas reacts to a certain extent with salt in the solution, a modified form of Henry's law sometimes holds. Thus Hufner (137) found that nitric oxide dissolved in ferrous salt solutions in accordance with the equation

$$\alpha = a + bP_g \quad (15)$$

The pressure range 550 to 710 mm. was covered at 20°C., Neuhausen and Patrick (225, 226) proposed the use of an adsorption equation for solubility

$$\alpha = a \left(\frac{\sigma P_g}{P_0} \right)^{1/n} \quad (16)$$

in which σ is the surface tension and P_0 is the vapor pressure of the liquefied gas. It satisfied their own data for the system ammonia-water, and those of others for hydrogen chloride, carbon dioxide, and sulfur dioxide in water and in alcohols. The equation does not apply to gases above the critical temperature.

The work of Frolich and coworkers (94a) on the extension of Henry's law to high pressures is of importance in engineering calculations. The solubility of hydrogen, nitrogen, oxygen, and of methane and other hydrocarbons in water, alcohols, hydrocarbons, and heavier petroleum fractions was studied up to 200 atmospheres. They concluded that, when the gas does not form a chemical compound with the solvent, it follows Henry's law over a wide pressure range within the limits of error allowed in engineering calculations. The solubilities of these gases may be considered linear functions of the absolute pressure, the validity being dependent upon the extent to which the solute obeys the ideal gas law. However, the straight-line relationship still holds at high pressures, provided corrections are applied for deviations from the ideal gas law. A practical rule is that the solubility of a gas of the vapor type is a linear function of pressure up to one-half to two-thirds of its saturation value at that temperature.

C. VARIATION OF SOLUBILITY WITH TEMPERATURE

Bunsen (37, 38, 39, 40) applied a purely empirical equation to the data that he found for the solubilities of a number of gases in water and alcohol. The equation had the form

$$\alpha = a + bt + ct^2 \quad (17)$$

Numerous others applied similar formulae. Thus Carius (44), Than (306), and Timofeev (312) applied the same equation to their data. The constants are found by substituting experimental values at three temperatures into the equation. In most cases the solubility decreased with increase in temperature, although the solubility of hydrogen in water was constant. Winkler (336) added a term in t^3 to his equation. Henrich (117) used Bunsen's data to recalculate the constants, using the method of least squares. Wiedeman (333) showed that, while the values of a , b , and c for the different gases in water were very different, the ratios b/a and c/a were nearly the same for all gases. The same was found for alcohol. Fox (93) and Whipple and Whipple (332) applied the same type of equation to atmospheric gases in distilled water and sea water. The latter also added similar terms to include the chlorinity of sea water. These equations were all purely empirical and usually were applied over a rather limited range of temperature.

Bohr (24) proposed that at constant partial gas pressure the osmotic pressure of a dissolved gas is constant. Thus αT is constant. When he substituted values, however, he found that such was not the case, but that

$$\alpha (T - a) = k \quad (18)$$

He found a to be a constant which, for five diatomic gases (hydrogen, nitrogen; oxygen, carbon monoxide, nitric oxide) in water, was a linear function of the molecular weight. These relations seem to have been at first purely empirical. In a later article (25) he made an effort to establish a theoretical basis for his first equation. He equated the rate of solution of a gas at equilibrium to its rate of escape, and made measurements of each. He established an empirical relation for the rate of escape, which proved to give the equation that he sought. The empirical nature of his result, however, remained. Later Kofler (161) showed that the same equation fitted his results for the solubility of radon in water, over the range 0° to 75°C .

Kofler (162) stated that there is a connection between the critical temperature of a gas and its solubility in a given solvent. He plotted T/T_c for a number of gases against α in water, and found that they fell on a smooth curve.

Meyer (208) applied the equation

$$S = b + e^{-a\theta} \quad (19)$$

to the solubility of various gases in various solvents. S can be replaced by either the Bunsen or the Ostwald coefficient, with corresponding values for the constants. However, the equation fits better if it refers to the amount of gas dissolved in a unit weight of solvent. θ is a measure of the temperature, on a scale such that for a given solvent there are 100 degrees between the melting point and the boiling point. In the case of water it is the Centigrade scale. Meyer found a to be nearly the same for all gases and all solvents. For the system radon-water, the equation fits the results very well.

Jager (139), from kinetic considerations, derived the equation

$$L = e^{-\frac{A}{RT}} \quad (20)$$

Empirically he found that

$$A = a (1 + bt (1 - ct)^2) \quad (21)$$

Using values from the literature for several gases in water, he calculated the values of the constants, and found that the equation was satisfied within 2 or 3 per cent. The constant c proved to be nearly constant for all gases, and equal to the temperature coefficient of the capillarity con-

stant of water. Szeparowicz (302) applied Jager's formula as well as that of Meyer (208) to his data for the solubility of radon in water, which he had carried up to 100°C., and found that both formulae were satisfied. Jager's equation also satisfied his data for radon in benzene.

The Clapeyron equation has been used as a basis for the derivation of several equations relating gas solubility and temperature. If the heat of solution of the gas in the liquid is constant, and is not a function of temperature over the interval used, the Clapeyron equation gives:

$$\ln \frac{L_1}{L_2} = -\frac{A}{R} \left(\frac{1}{T_1} - \frac{1}{T_2} \right) \quad (22)$$

Gas solubility also may be expressed as K_1 , α or S in this equation. Graphically $\log L$ is a linear function of $1/T$. This equation more frequently has been expressed in the exponential form:

$$L = ae^{-\frac{A}{RT}} \quad (23)$$

Both Tammann (304) and Lannung (172) have used it in this form, which may be compared with the equation of Jager (equation 20). Lannung, using his data for the rare gases, found that $\log L$ was a linear function of $1/T$ for the organic solvents used but not for water. There was an approximately linear relationship between A and $\log a$. Using the data of Markham and Kobe (201) for the solubility of carbon dioxide in aqueous salt solutions from 0 to 40°C., a maximum deviation of 3 per cent existed for water at 25°C. The salt solutions all showed smaller deviations, ranging down to 0.85 per cent for 3 molal magnesium nitrate solution.

If the heat of solution of the gas in the liquid is a function of the temperature, then an equation of the form of the reaction isochor results, which may be shortened to the form used by Valentiner (322):

$$\log L = \frac{a}{T} + b \log T + c \quad (24)$$

This equation fits the solubility data for the inert gases in water.

The general rule is that the solubility of a gas in water decreases with increasing temperature. However, the Bunsen absorption coefficient for hydrogen reaches a minimum at 60°C. with no further change to 100°C.; for nitrogen the minimum is at 90°C., and for helium it is at 30°C. with a marked increase up to 100°C. If, instead of the Bunsen coefficient, the Ostwald coefficient of solubility is used, the minima in the curves come at much lower temperatures and other gases show increasing values of L with rise in temperature. With helium the minimum is below 0°C. At pressures up to 1000 atmospheres this minimum in the solubility isobar

becomes quite apparent; it has been studied by Wiebe, Gaddy, and co-workers (332a). Above 200 atmospheres partial pressure, carbon dioxide shows an increase in the absorption coefficient in water. In non-aqueous solvents an increase in solubility with rise in temperature is a common phenomenon. Lannung, Horiuchi, and others have shown the increased solubility with rise in temperature of the relatively insoluble gases. The effect is usually small at atmospheric pressure but may become quite large at higher pressures, as indicated by the sevenfold increase in the solubility of hydrogen from 0° to 100°C. in liquid ammonia at 1000 atmospheres (332a).

With the Ostwald coefficient for a relatively slightly soluble gas, the concentrations of the gas in the liquid and the gaseous phases should approach the same value as the solvent approaches the critical temperature, or L approaches 1. Thus, all such gases should show a minimum in the solubility isobar at some definite temperature, from below 0°C. for helium to higher temperatures for other gases. Horiuchi took the critical temperature of the solvent (T_K) into consideration and found, by plotting $\log L$ against T_K/T that the solubility lines for a particular gas in a number of solvents fall closer together. However, it may be concluded that the minimum in the solubility isobar is not a peculiar property of the gas or of the solvent, but is a phenomenon of the mixture and may be predictable from known properties of mixtures.

D. VARIATION OF SOLUBILITY WITH CONCENTRATION

The effect of the addition of another solute on gas solubility has frequently been investigated, and several formulae have been proposed to express this effect. In the following discussion "solute" will refer to the soluble substance whose concentration is the independent variable.

Raoult (254) found that, when ammonia dissolved in water and aqueous salt solutions, the gas solubility was a linear function of the solute concentration. This is the simplest relation that could be desired. Hufner (136), who worked with hydrogen and nitrogen in aqueous solutions of organic compounds, also found a linear relationship. He further found that, in comparing some compounds with each other, the solubility lowering was proportional to the molar concentration, the proportionality factor being the same for different solutes. In the case of other compounds, however, he found that the lowering produced was proportional to the weight of solute per volume of solution. His experimental results were not very good, but seem to confirm the above relation fairly well. Hudson (135) found that the solubility of sulfur dioxide in aqueous potassium chloride solutions bore a linear relationship to the salt concentration. Such was not the case when sodium sulfate was the solute, however. Konovalov

(165) found that the pressure of ammonia dissolved in aqueous solutions of copper and silver salts satisfied the formula

$$P_o = P_{o_0}(C_o - aC_s) \quad (25)$$

in which C is the ammonia concentration and a is 2 for silver and 4 for copper. For other salt solutions, he found (166) that $(P_o - P_{o_0})/C_s$ increased as C_s increased. Abegg and Reisenfeld (1) found that the effect of salt on the pressure of ammonia was linear with the salt concentration, but that the solubility was not linear with salt concentration. Their results thus did not agree with those of Kononov.

Secenov (278, 279) introduced an equation that has been used frequently. He stated the hypothesis: "If equal quantities of the same salt are added to equal volumes of different aqueous solutions, the percentage reduction in solubility will be the same in both." This hypothesis was poorly supported by one experiment. If it is accepted, however, there results his equation:

$$\alpha = \alpha_0 \epsilon^{-kC_s} \quad (26)$$

Another equation frequently found in the literature is referred to as Jahn's equation (140), but appears to have been published first by Gordon (104). It is

$$\alpha_0 - \alpha = kC_s^{\frac{1}{3}} \quad (27)$$

The two-thirds power brings in some surface relation. Gordon's data were not very good and did not satisfy the equation very well.

From thermodynamic considerations Roth (262) derived the relation that the molecular concentrations of gas in pure water and in dilute solutions of inert substances, at the same temperature and partial pressure, are the same. Nitrous oxide in aqueous solutions of urea, oxalic acid, and glycerol satisfied his theory fairly well. Solutions of sodium chloride and phosphoric acid did not, however, but did satisfy Jahn's equation. The glycerol solution did not satisfy Jahn's equation, since the reduction in α was nearly proportional to the solute concentration.

Steiner (295) used his data on hydrogen in aqueous salt solutions to test Secenov's equation, and found that it did not hold well. Rothmund (263) showed that, from Secenov's equation

$$\log \frac{\alpha_0}{\alpha} = \log \frac{L_0}{L} kC_s \quad (28)$$

and in dilute solution,

$$\frac{L_0}{L} = kC_s \quad (29)$$

Tammann (304), from relations that he found empirically, arrived at Secenov's formula. Åkerlöf (3) found the values of k in Secenov's formula for helium and argon in aqueous salt solutions, but had insufficient data to make any verification. Kiss, Lajtai, and Thury (152), who worked with carbon dioxide and hydrogen sulfide in aqueous solutions of organic substances, found that neither Secenov's nor Jahn's equation was satisfactory. Calvet (43) made an effort to justify Secenov's formula from experimental data on the mobility of molecules in a solvent.

The data of Markham and Kobe (201) on carbon dioxide and nitrous oxide in water and in numerous aqueous salt solutions, over a wide range of concentration, satisfied none of these equations. They proposed the equation

$$\frac{S}{S_0} = am + \frac{1}{1 + bm} \quad (30)$$

This equation fits their data within the experimental error of 0.2 per cent. They showed further (201a), from data on sulfuric acid and perchloric acid solutions, that the equation held through a minimum in the solubility curve. However, the agreement does not extend to the maximum in the curve. This equation is that of a hyperbola, with a vertical asymptote at $m = -1/b$, the other asymptote having a slope of S_0a (figure 5). Most solubility curves are in the region of this equation in which the slope is negative, before the minimum is reached.

Braun (30) determined the solubility of nitrogen and of hydrogen in aqueous solutions. He was satisfied with Roth's formula for solutions of urea and propionic acid, although in 10 per cent urea solutions there were deviations from the formula of as much as 10 per cent. For sodium and barium chlorides he used Jahn's equation, in which the maximum deviation was about 2 per cent. Levi (177) experimented with solutions of potassium iodide and urea in methanol, and reported that Jahn's formula held, while Roth's also held for the urea solution. Locke and Forssall (183) used Jahn's formula in their determination of the amount of ammonia in the copper ammonia complex in solution. Knopp (157) used his data on hydrogen and nitrous oxide in aqueous salt solutions to test Jahn's formula as well as Roth's. Neither was satisfactory. His values, however, satisfied another formula derived by Jahn (140), namely:

$$\log \frac{C_i}{C_{i_0}} = C_i(1 - f)(a + fb) \quad (31)$$

in which f is the degree of dissociation as found by conductivity, and C_i and C_{i_0} are the molecular concentrations of gas in solution and in pure

water, respectively. If a is small and f is nearly constant, then the above formula reduces to

$$\log \frac{C_l}{C_{l_0}} = C_s k \quad (32)$$

This formula was not satisfactory.

Usher (320) worked with carbon dioxide and aqueous solutions of non-electrolytes. He claimed a high degree of accuracy for his data, which probably were much more of a test of the formulae than most that had

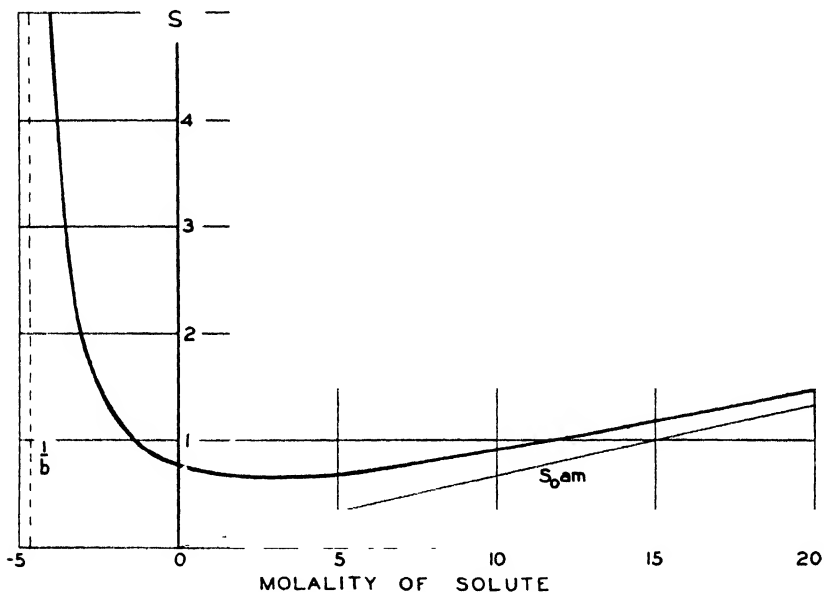


FIG. 5. Solubility isotherm for equation 30. Constants from carbon dioxide in aqueous sulfuric acid.

been used. He felt that the formulae of Jahn and Roth were of little value, since he showed that frequently the deviation from theory is greater than the effect to be explained.

Philip (243) made two suggestions intended to bring into better agreement the solubility of gases in solutions: first, that all solubilities be expressed on a basis of 1000 g. of solvent, i.e., water in an aqueous solution, rather than on a volume of solution; and second, that the loss of solvent to solvate the solute accounts for the reduction in gas solubility when the weight basis is used. MacArthur (190) used his data on oxygen solubility to find the degree of hydration of a number of salts and of sucrose. The

values that he found were consistent with those found by other methods. Manchot (198), from data on the solubility of nitrous oxide and acetylene, calculated the degree of hydration of a number of salts. The two gases gave similar results. Gaus (99) found an apparent connection between the atomic volume of the cation of a salt and the effect of the salt on the partial pressure of ammonia. Usher (320) abandoned the hydration hypothesis to account for the change in solubility in solutions of non-electrolytes, since in several cases the solubility was increased by the addition of solute. Perman (239, 240, 241) found that the addition of urea to an aqueous ammonia solution caused little change in the pressure of ammonia, while mannitol and several salts caused somewhat more change.

Jones, Lapworth, and Lingford (145) used the Duhem equation to express their results on the partial pressure of hydrogen chloride over water-alcohol solutions. Intermediate empirical equations enabled them to effect the integration, giving the result

$$\log P_g = a \log y + by + cy^2 + k \quad (33)$$

in which y is the moles of hydrogen chloride per mole of alcohol. They expressed the constants as functions of the water content of the solution in a purely empirical way.

Randall and Failey (253), using examples from the literature, found that plots of $(\log \gamma)/\mu$ against $\sqrt{\mu}$ gave straight lines. In most instances these lines were horizontal. Markham and Kobe (201) confirmed this relation for their data on the solubility of carbon dioxide and nitrous oxide in aqueous salt solutions. They further showed, from differentiation of equation 30, that

$$\frac{\ln \gamma}{m} = b - a \quad (34)$$

at low concentrations, in agreement with Randall and Failey.

E. GENERAL RELATIONSHIPS

1. Additive effect of ions

Steiner (295) found that, in dilute solutions of several strong electrolytes, the reduction in the solubility of hydrogen was an additive function of the ion concentrations. Van Slyke and Sendroy (326) found the same result for carbon dioxide and hydrogen in aqueous solutions of alkali chlorides, lactates, and phosphates. Markham and Kobe (201) found a similar result for the solubility of carbon dioxide in aqueous solutions of the chlorides and nitrates of sodium and potassium, in concentrations up to 1 molal.

2. *Specific effect of solute*

Rothmund (263), Euler (78), and McLauchlan (205) arranged numerous salts in the order of the percentage lowering of the solubility that they produced, and all found the same order. The "gases" used were phenylthiourea, ethyl acetate, and hydrogen sulfide, respectively. Reisenfeld (256) found that the equivalent solubility lowering of all salts (as they affected ammonia) was the same, barring specific chemical action. This question of chemical action has come up repeatedly. Secenov (279) stated that all salts take an active part in the absorption of carbon dioxide. Others have tried to distinguish between the chemical and the physical effects of solutes on the solubility of a gas. Rothmund (263) in this connection found that $(L_0 - L)/L_0$ was independent of temperature, and from this relation and the Clapeyron equation showed that the heat of solution of phenylthiourea in water and in salt solution was the same, indicating the absence of chemical reaction. Bell (15), from his data on the solubility of hydrogen sulfide, ammonia, and hydrogen chloride in a number of solvents, believed that the solubility depended on individual properties of the solvent molecule.

Drucker and Moles (71) plotted several properties of the solution against the composition of aqueous glycerol solutions. The properties were: heat of solution, coefficient of expansion, surface tension, specific heat, and the solubility of nitrogen and hydrogen. These properties deviated from the straight line that would result in the case of perfect solutions. The point of maximum deviation of the properties fell at the same composition, except in the case of solubilities. From the fact that these fell at different points, Drucker and Moles concluded that solubility depends on the chemical properties of the solvent, rather than on physical properties.

Skirrow (289) found the solubility of carbon monoxide in several mixtures of organic solvents. In several cases the solubility was an additive function of the solvent concentrations, but usually it was not. Some solutions showed a minimum in the surface tension-composition curve and a maximum in the solubility curve at nearly the same concentration. Christoff (49) found the same result in the solubility of carbon dioxide in several solutions.

3. *Effect of surface tension*

Christoff (50) measured the solubility of several gases in ether, which has an extremely low surface tension, to show that some relation existed between the two. Gases proved to be more soluble in ether than in other solvents with which comparison was made. Uhlig (318) considered the energy change in transferring a gas molecule from the gas phase into the liquid against the force of surface tension, and derived the equation

$$\ln L = \frac{-4\pi r^2 \sigma + E}{KT} \quad (35)$$

in which r is the molecular radius of the gas, E is the interaction energy, σ is the surface tension of the solution, and K is Boltzmann's constant. E and r , found from solubility data, checked the same quantities found by other means. Eley (75), in the first of a series of articles, proposed the mechanism of cavity formation, after which the gas molecule enters the cavity. At the temperature of maximum density, the energy and entropy of cavity formation are zero, but increase with increase in temperature.

Sisskind and Kasarnovskii (288) measured the solubility of argon in various organic solvents, including several homologous series. Tables were given of the solubility of the gas, the molecular volume of the liquid, the surface tension, the dipole moment, and the polarizability of the solvents. The solubility, the surface tension, and the molecular volume were in substantially the same order.

4. Effect of viscosity

Winkler (339, 342) proposed an equation relating solubility to viscosity. Elsewhere this equation is credited to Than. This equation can also be considered as a relation between solubility and temperature, since the viscosity change with temperature is the variable. The equation is

$$\frac{\alpha_1 - \alpha_2}{\alpha_1} = \frac{Z_1 - Z_2}{Z_1} \frac{\sqrt[3]{M}}{k} \quad (36)$$

in which Z is the viscosity and the subscripts refer to values at two temperatures. For five fixed diatomic gases in water, K proved to be nearly equal to the cube root of 54, three times the molecular weight of water. The data that Winkler gave showed remarkable agreement with the equation. Thorpe and Rodgers (309) stated that Winkler's conclusions must be changed to: "For the same gas, the decrease in solubility (not percentage decrease) is proportional to the corresponding decrease in viscosity; and further, for any gas, the factor of proportionality is greater for a greater molecular weight, but no simple relation exists."

Winkler stated that with the increase of the volume of the solvent with temperature, the coefficient should increase, but that the decrease in the viscosity should cause a tendency for the coefficient to decrease. The result should be a minimum in the temperature-solubility curve. Such a minimum has been observed in several cases, as well as a positive temperature coefficient of solubility in others.

5. Homologous compounds

Just (147) arranged a number of organic solvents in the order of their ability to dissolve each of several gases, and found the order to be nearly

the same for all the gases that he used. When so arranged, the compounds of each series having a common reactive group fell in the order of their molecular weights, with the solubility decreasing as the molecular weight increased. He found the solubility ratios of two gases in the same liquid to be of the same order of magnitude for all liquids. Horiuchi (130, 131, 132, 133) found this to be true only for low-boiling gases. McDaniel (204) found that the solubilities of three gaseous hydrocarbons increased in the same order in the liquids with which he experimented. Sander (266) found that in homologs the solubility of carbon dioxide decreased with increasing molecular weight. Korosy (167) found that different gases in one solvent fit approximately a formula equivalent to

$$L = a + bT_c \quad (37)$$

a and b being constants of the solvent, and b nearly the same for all solvents. Markham and Kobe (201) used Duhring lines to express the solubility of carbon dioxide and nitrous oxide in various aqueous salt solutions. They showed that, for any salt, if the concentration at which the gas solubility was the same as for a certain concentration of a reference salt was plotted against the concentration of the reference salt, a straight line resulted (figure 6). Deviations, though greater than experimental error, were still not over several per cent.

6. *Effect of the compressibility of the liquid*

Ritzel (257) related gas solubility to the compressibility of the liquid. He derived the relation:

$$L = \frac{P_g B}{\delta} \quad (38)$$

in which δ is the coefficient of dilatation, and B is the compressibility of the liquid. Accepting Ångström's (4) conclusion that the ratio of the coefficients of dilatation for two gases in one liquid is independent of the liquid, it follows that the ratio of the solubility of two gases in one liquid is nearly the same for all liquids, as Just found. Kofler (162) arranged a number of salts in the order of their ability to decrease the solubility of phenylthiourea in water, and found nearly the same ratio as that of their ability to decrease compressibility. He plotted various properties of aqueous sulfuric acid solutions against concentration,—e.g., viscosity, compressibility, volume contraction on mixing, conductivity, and the solubility of nitrogen and hydrogen. The curves of solubility and compressibility were similar. Horiuchi (134) found a relationship between

partial molal volume, the solubility of a gas, and the compressibility of a liquid. It was satisfactory for hydrogen in carbon tetrachloride, but not for other systems in which comparison was made.

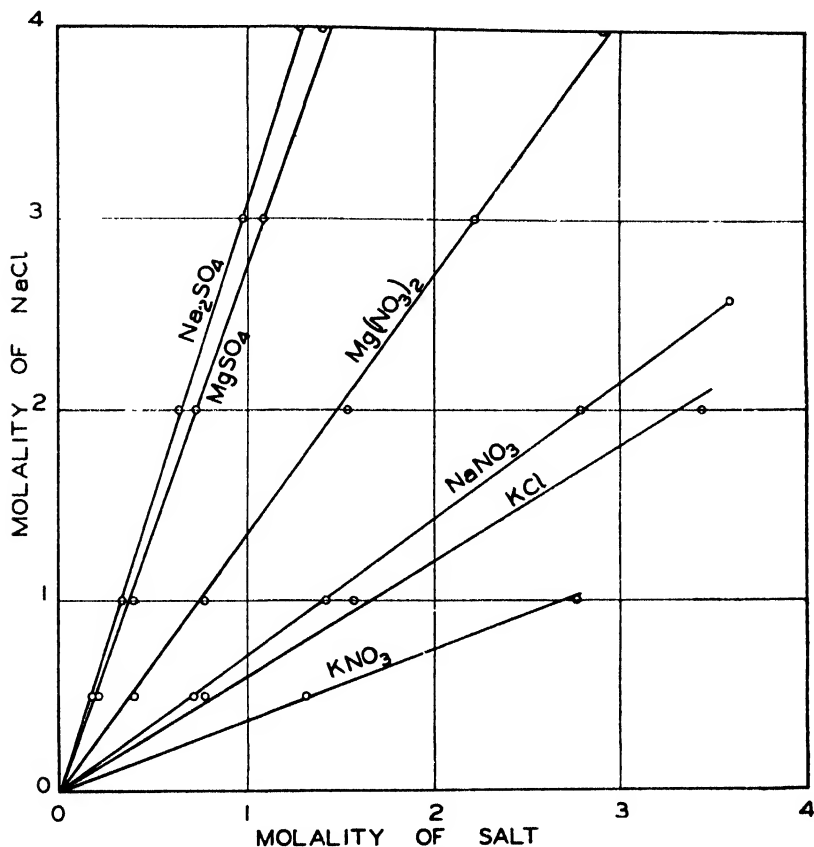


FIG. 6. Dühring lines of equal gas solubility, using sodium chloride solution as the reference solution.

7. Relationships from Raoult's law

Dolezalek (68) derived relationships of solubility based on Raoult's law. From his expression

$$L = \frac{X_i}{1 - X_i} \frac{1000\rho}{C_g M_g} \quad (39)$$

he calculated solubility in several organic liquids, and checked Just's data within 5 to 20 per cent. This relation, in the case of gases the solu-

bility of which is small, readily reduces to Just's conclusion that the ratio of the solubilities of two gases in the same liquid is independent of the liquid. In the case of nitrogen and carbon monoxide, Dolezalek verified Just in this respect. This pair is the only satisfactory one that Just found for this comparison, and these gases are isoelectronic. Stern (297) used Just's data for the verification of Dolezalek's equation, and showed that, if association of the liquid were used to explain discrepancies, as Dolezalek suggested, then unreasonable degrees of association were found. Schulze (272) tested Dolezalek's theory, using values from the literature for the solubility of radon in organic liquids. The curves of solubility that he found thus were of the same general form as the experimental curves, but far off in values.

8. Effect of the internal pressure of the liquid

Euler (78) suggested that the decrease in gas solubility caused by a solute was due to the increase in internal pressure in the solution. He used the equivalent contraction accompanying solution as a measure of the internal pressure increase, and found that, in the case of the salts that he used, the lowering of the solubility of ethyl acetate was in the same order as the equivalent contraction.

Geffcken (100) mentioned the possible relation between gas solubility and the internal pressures of gas and liquid. Hildebrand (121) calculated the theoretical solubilities of several gases, based on Raoult's law, and compared the values with those found in the literature for a number of solvents. He stated that deviations from the value predicted by Raoult's law were large or small depending on the difference in the internal pressures of the gas and liquid, except in the case of highly polar pairs. Taylor and Hildebrand (305) used experimental data of their own on chlorine in several solvents in proceeding with the same idea. Kunerth (171) questioned the value of this theory, but, within the limitations proposed by Hildebrand himself, the data support the theory. Hildebrand (122, 123) restated his theory and its limitations as follows: "Raoult's law will be obeyed by any liquid mixture in which the internal forces of attraction and repulsion do not change with changing composition of mixture. When this condition holds the solubility of a gas may be calculated approximately from its saturation pressure, and the solubility of a solid from its melting point and heat of fusion. The above condition can exist only (a) when the components in the pure liquid phase have the same internal pressures; (b) when the different molecules are relatively symmetrical or non-polar; (c) when the tendency to form compounds is absent. Differences in either internal pressure or polarity alone produce approx-

imately proportional positive deviations from Raoult's law and decreased solubilities. . . ."

Hamai (109, 110) found that his data on the solubility of hydrogen chloride in several organic halogen compounds did not correlate with their internal pressures or polarity, but varied in the same order as their total bond energy.

9. *Miscellaneous*

Homfray (128) found the solubility of carbon dioxide in *p*-azoxyphenetole in both the liquid crystal and the anisotropic states, and showed that the state of the solvent had considerable effect on the solubility.

Sackur (265) used gas solubility data to find the osmotic pressure of the gas in the liquid phase, and found that the results so calculated agreed with the experimental within a few per cent.

Bell (14) found that for the solubility of gases a linear relation existed between the energy and the entropy of solution of different solutes in the same solvent.

IV. SOLUBILITY DATA

In this section reference is made to all available data for a particular gas, giving the solvent employed, the range of temperature and pressure, and the reference to the original literature. An effort has been made to indicate the probable reliability of the data, on the basis of the method employed, the completeness of the data, and the consistency of the results among themselves. Comparison among the various workers in general has not attempted. Numbers ranging from 4 to 1 are found in the column headed "Value", in which a value of 4 indicates data in which considerable reliance can be placed, although comparison of these values as given by different experimenters reveals discrepancies in some cases. The smaller values indicate less reliable data; number 1 indicates data which are little more than qualitative.

TABLE 1
Solubility data

GAS	SOLVENT	PRESSURE	TEMPER- ATURE	VALUE	REFER- ENCES
°C.					
A. Inert gases 1 Helium	Water	Atm.	2-30	4	(41)
		Atm	25-75	4	(332a)
		Atm	25	4	(3)
		Atm	15-37	4	(172)
		1 to 6 atm	38	4	(115)
		Atm.	0-50	3	(5)
		Atm.	0-50	2	(77)
		Atm.	18	1	(251)
			0-45	1	(321, 323)
		Methanol	Atm	15-37	4
	Cyclohexanol	Atm	25-37	4	(172)
	Benzene, cyclohexane	Atm	15-37	4	(172)
	Acetone	Atm	15-25	4	(172)
	Blood	1 to 6 atm.	38	4	(115)
	Aqueous solutions				
Solute KCl, NaCl, LiCl, LiI, NaNO ₃ , HClO ₄	Atm.	25	4	(3)	
2 Neon	Water	Atm	15-37	4	(172)
		Atm.	0-50	3	(5)
		Atm.	0-45	1	(321, 323)
	Methanol	Atm	15-37	4	(172)
	Cyclohexanol	Atm	25-37	4	(172)
		Atm.	25	4	(46)
	Benzene, cyclohexane	Atm.	15-37	4	(172)
	Acetone	Atm	15-25	4	(172)
3 Argon	Water	Atm.	25	4	(3)
		Atm.	15-37	4	(172)
		Atm.	0-50	3	(5)
		Atm.	0-50	2	(77)
		Atm.	12	1	(250)
		Atm.	2-25	2	(249)
	Sea water	Atm.	15-37	4	(172)
	Methanol	Atm.	25-37	4	(172)
	Cyclohexanol	Atm.	26	4	(46)
	Benzene, cyclohexane, acetone	Atm	15-37	4	(172)
	Chloroform		Room	2	(167)
	Paraffin oil		32		(222)
	Aqueous solutions*				
Solute. KCl, NaCl, LiCl, NaNO ₃ , CaCl ₂ , SrCl ₂ , BaCl ₂ , MgCl ₂ , AlCl ₃	Atm.	25	4	(3)	
4 Krypton	Water	Atm.	0-50	3	(5)
		Atm.	Room	3	(180)
		Atm.	Room	3	(180)
		Atm.	Room	3	(180)
		Room	2	(167)	
	Amyl alcohol	Atm.	Room	3	(180)
	Glycerol	Atm.	Room	3	(180)
		Room	2	(167)	
	Cyclohexanol		Room	2	(167)
	Acetic acid		Room	2	(167)

TABLE 1—*Continued*

GAS	SOLVENT	PRESSURE	TEMPER- ATURE	VALUE	REFER- ENCES
<i>A. Inert gases—Continued:</i> 4 Krypton			°C.		
	Butyl acetate, butyl phthalate, tricresyl phosphate, acetone, tetralin, bromoform		Room	2	(187)
	Benzene	Atm.	Room	3	(180)
	Toluene, xylene		Room	2	(187)
	Petroleum fractions	Atm.		3	(180)
	Chloroform, carbon tetrachloride		Room; 0	2	(187)
	Calcium chloride solution (aqueous)		Room	2	(187)
5. Xenon	Water	Atm.	0-50	3	(5)
<i>B. Elementary gases</i> 6 Hydrogen.	Water	750 to 1400 mm.	25	4	(86)
		Atm.	25	4	(71)
		Atm.	0-60	4	(339)
		Atm.	0-100	4	(337)
		Atm.	0-20	3	(312)
		Atm.	20	3	(157)
		Atm.	20-25	3	(147)
		Atm.	15	3	(221)
		700 mm.	20	3	(136)
		Atm.	20	3	(49)
		Atm.	25	3	(100)
		Atm.	5-25	3	(30)
		Atm.	0-20	2	(37, 38, 39, 40)
		Atm.	20-25	2	(207)
		Atm.	25	2	(212)
		Atm.	20	2	(185)
		Atm.	15-30	2	(275)
		Atm.	11-19	2	(295)
		900 to 8200 mm.	20-25	2	(45)
	Methanol	Atm.	20-25	3	(147)
	Ethanol	Atm.	0-50	4	(202)
		Atm.	20-25	3	(147)
		Atm.	0-20	3	(312)
		Atm.	20	3	(49)
		Atm.	0-25	2	(44)
		Atm.	0-20	2	(40, 117)
		Atm.	25	3	(147)
	Propyl alcohol	Atm.	20-25	3	(147)
	Amyl alcohol	Atm.	25	4	(46)
	Cyclohexanol	Atm.	20-75	4	(202)
	Acetic acid	Atm.	20-25	3	(147)
		Atm.	-80-+40	4	(133)
	Methyl acetate	Atm.	0-40	4	(202)
	Ethyl acetate	Atm.	20-25	3	(147)
		Atm.	20-25	3	(147)
	Isobutyl acetate, amyl acetate	Atm.	10-40	4	(202)
	Benzene	Atm.	7-63	4	(133)
		Atm.	20-25	3	(147)
	Toluene, xylene	Atm.	20-25	3	(147)

TABLE 1—*Continued*

GAS	SOLVENT	PRESSURE	TEMPERATURE	VALUE	REFERENCES
			°C.		
<i>B Elementary gases</i> —Continued: 7. Nitrogen	Blood, blood fluids Liquid oxygen Liquid sulfur dioxide Sulfuric acid Aqueous solutions. Solute: NaCl, Na ₂ CO ₃ H ₂ SO ₄ Urea, propionic acid, BaCl ₂ , NaCl Glycerol, isobutyric acid Glycerol, chloral hydrate Sucrose, dextrose, glycerol, chloral hydrate Urea, arabinose, glycocoll, acetamide, dextrose, levulose, alanine Acidified sodium sulfate solution Dyes Non-aqueous solutions: Methanol solutions of urea, KI	1 to 6 atm.	38	4	(114)
		Atm.	23	4	(55)
		Atm.	37 5	4	(105)
			—190	1	(76)
		100 to 700 mm.	—60—20	2	(69)
		Atm.	20	2	(49)
		Atm.	38	4	(325)
		Atm.	Room	3	(27)
		Atm.	5-25	3	(30)
		Atm.	25	3	(71)
		Atm.	15	3	(112)
		Atm.	15	3	(221)
		700 mm.	20	3	(136)
		Atm.	25	4	(158)
			14.5	2	(331)
					(177)
8. Oxygen.	Water	175 to 760 mm.	25	4	(218)
		Atm.	25	4	(216)
		Atm.	0-60	4	(339)
		Atm.	0-100	4	(338)
		550 to 800 mm.	0-50	4	(93)
		Atm.	25	3	(100)
		Atm.	20	3	(49)
		Atm.	0-20	3	(65)
		Atm.	15	3	(221)
			0-14	3	(242)
		Atm.	6-12	3	(312)
		Partial pressure in normal air	0-30	3	(382)
		Partial pressure in normal air	0-30	3	(335)
		Partial pressure in normal air	25	3	(190)
		900 to 8200 mm.	20-25	2	(45)
		Atm.	0-25	2	(37, 38, 39, 40)
		Atm.	20	2	(185)
		Atm.	18	2	(192)
		Atm.	20-25	2	(207)
		Atm.	15-80	2	(275)
		Atm.	5-25	1	(281)
			0-100		(70)
Sea water					(47)
			0-28	3	(93)

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPER- ATURE	VALUE	REFER- ENCES
			°C.		
<i>B. Elementary gases</i> —Continued: 8. Oxygen ..	Sea water	Partial pressure in normal air	0-30	3	(332)
			2-35	From litera- ture	(2)
	Ethanol	Atm	6-23	3	(312)
		Atm.	20	3	(49)
		Atm.	20-25	2	(207)
		Atm.	0-25	2	(40)
		Atm.	0-25	2	(44)
	Cyclohexanol	Atm.	25	4	(46)
	Methyl acetate	Atm.	-80 +40	4	(133)
	Benzene	Atm	10-60	4	(133)
		Atm	25	4	(217)
	Carbon tetrachloride	Atm.	0-60	4	(133)
		Atm.	20-25	2	(207)
	Acetone	Atm.	-80 +40	4	(133)
		Atm	20-25	2	(207)
	Diethyl ether	Atm.	-80 +20	4	(133)
		Atm.	0-15	4	(50)
	Chlorobenzene	Atm.	-40 +80	4	(133)
	Petroleum fractions	Atm.	20	3	(49)
		Atm.	2-25	2	(170)
		Atm.	10-20	2	(101)
		Atm.	20		(194)
	Sulfuric acid	Atm	20	3	(49)
	Cottonseed oil, corn oil, lard		23-45		(327)
	Blood	Partial pressure in normal air	39	1	(138)
	Liquid sulfur dioxide	100 to 700 mm	-60--20	2	(69)
	Aqueous solutions.				
	Solute:				
	Sucrose, dextrose, glycerol, chloral hydrate	Atm.	15	3	(221)
	Ethanol	Atm	20	2	(117)
	H ₂ SO ₄	Atm.	Room	2	(27)
	HCl, HNO ₃ , H ₂ SO ₄ , NaCl, K ₂ SO ₄ , KOH, NaOH	Atm.	15-25	3	(100)
	Sucrose, LiCl, NaCl, KCl, RbCl, CsCl, NaBr, KBr, KI, KNO ₃ , Na ₂ SO ₄ , K ₂ SO ₄ , MgCl ₂ , CaCl ₂ , BaCl ₂	Partial pressure in normal air	25	3	(190)
	NH ₄ Cl	Partial pressure in normal air	25	1	(190)
		Atm.	25	3	(59)
	KCN	Atm.	18	2	(192)
	Acidified sodium sulfate so- lution	Atm.	25	4	(158)
	Gas-main condensate		0-100		(70)
9. Ozone .	Water	About 2 to 70 mm.	0-60	3	(31)
			0-60	1	(193)
	Carbon tetrachloride	About 70 mm.	-12-0	3	(32)

[illegible]

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPERATURE	VALUE	REFERENCES
<i>B. Elementary gases</i>					
—Continued:					
11 Air	Ethanol, petroleum fractions, H ₂ SO ₄	Atm.	20	3	(49)
	Cottonseed, herring, cod-liver, maize, linseed, olive, and mineral oils	Atm.	20-50		(268)
<i>C. Compound gases.</i>					
12. Methane	Water	Atm.	0-100	4	(340)
		Atm.	20	3	(49)
		Atm.	0-25	2	(37, 38, 39, 40)
	Methanol	Atm.	20		(90)
		Atm.	20-50	4	(204)
		Atm.	20-40	4	(204)
	Ethanol	Atm.	20	3	(49)
		Atm.	0-25	2	(40)
		Atm.	0-25	2	(44)
	2-Propanol ("isopropanol")	Atm.	20-60	4	(204)
	Amyl alcohol	Atm.	20-30	4	(204)
				1	(94)
	Cyclohexanol	Atm.	25	4	(46)
	Isopentane	2200 to 7600 mm	30	2	(245)
	Benzene		10-60	4	(133)
			Atm.	4	(207)
	Toluene	Atm.	25-60	4	(204)
	m-Xylene	Atm.	20-60	4	(204)
	Hexane	Atm.	20-40	4	(204)
	Pinene	Atm.	20-55	4	(204)
	Diethyl ether	Atm.	0-15	4	(50)
		Atm.	-80 + 20	4	(133)
		Atm.	20		(90)
	Acetone, methyl acetate	Atm.	-80 + 40	4	(133)
	Carbon tetrachloride	Atm.	25	4	(134)
		Atm.	-20 + 60	4	(133)
		Atm.	20	3	(49)
	Sulfuric acid	Atm.	-40 + 100	4	(133)
	Chlorobenzene	Atm.	20	3	(49)
	Petroleum fractions	Atm.	10-20	2	(101)
		5100 to 6300 mm.	30	2	(245)
			20		(90)
	Acidified aqueous sodium sulfate solution	Atm.	25	4	(158)
13. Ethane	Water	Atm.	0-100	4	(340)
		Atm.	0-20	2	(267)
		Atm.	0-25	2	(37, 38, 39, 40)
				1	(270)
				4	(46)
	Cyclohexanol	Atm.	25		(46)
	Benzene	Atm.	0-50	4	(131, 133)
	Chlorobenzene	Atm.	0-80	4	(131, 133)
	Carbon tetrachloride	Atm.	0-40	4	(131, 133)
	Acidified aqueous sodium sulfate solution	Atm.	25	4	(158)

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPERATURE °C.	VALUE	REFERENCES
<i>C. Compound gases— Continued:</i> 14. Propane	Cyclohexanol Ethanol, ether, benzene, chloroform, essence of terebenthine	Atm.	25	4	(46)
		Atm.	Room	1	(175)
15. Butane	Water	Atm.	0-25	2	(37, 38, 39, 40)
16. Ethylene	Water	Atm.	25	4	(230)
		550 to 1000 mm.	25-37 5	4	(105)
		Atm.	15-80	2	(275)
		Atm.	15	2	(28)
		Atm.	0-25	2	(37, 38, 39, 40)
				1	(17)
					(227)
		Atm.	0-25	2	(40)
		Atm.	0-25	2	(44)
				1	(317)
	Ethanol			1	(17)
		Atm.	25	4	(46)
	Cyclohexanol	Atm.	25	4	(46)
	Acetone			1	(317)
	Benzene	Atm.	10-50	4	(131, 133)
		Atm.	20-50	4	(204)
	Xylene	50 to 750 mm.	-21-+40		(150)
	Carbon tetrachloride	Atm.	0-40	4	(131, 133)
	Chlorobenzene	Atm.	0-90	4	(131, 133)
	Petroleum fractions	Atm.	10-20	2	(101)
		50 to 750 mm.	-21-+40		(150)
	Blood fluids	550 to 1000 mm.	25-37 5	4	(105) (227)
17. Propylene .	Water	Atm.	0-20	2	(306)
				1	(17)
				1	(17)
				1	(150)
18. Cyclopropane	Vegetable, animal, and mineral oils				(231)
19. Isobutylene.	Xylene, petroleum fractions	50 to 750 mm	-21-+40		(150)
20. Acetylene	Water	Atm.	25	4	(198)
		Atm.	37.5	4	(105)
		Atm.	15	3	(23)
			25	3	(169)
			12-20	2	(220)
		Atm.	15-80	2	(275)
			0		(97)
				1	(317)
			18	1	(18)
		50 to 750 mm.	0		(151)

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPER- ATURE	VALUE	REFER- ENCES
			°C.		
<i>C. Compound gases—</i>					
Continued:					
20. Acetylene .					
	Amyl alcohol		18	1	(18)
	Cyclohexanol	Atm.	25	4	(46)
	Aniline		-6		(96)
	Dimethylaniline		2		(96)
	Cyclohexane		6		(96)
	Nitrobenzene		5		(96)
	Benzene	Atm.	10-45	4	(133)
			18	1	(18)
			5.5		(97)
	Acetaldehyde, propionalde- hyde, butyraldehyde, meth- ylal, acetal, methyl formate, ethyl formate, isoamyl for- mate, methyl acetate, ethyl acetate, isoamyl acetate, eth- yl mustard oil, acetoacetone, ethylidene cyanohydrin, methyl propyl ketone	Atm.	-10	2	(142)
	Carbon tetrachloride	Atm.	0-40	4	(133)
			18	1	(18)
	Chlorobenzene	Atm.	10-45	4	(133)
	Formic acid		7		(97)
	Acetic acid		15		(97)
	Bromoform		7		(97)
	Acetophenone		16		(97)
	Acetone		25	3	(169)
				1	(317)
		Atm.	15-50	1	(52)
		1.3 atm.	-80	1	(51)
			-20 +40		(210)
	Ethylene dichloride	50 to 760 mm.	0		(151)
	Pentane, carbon disulfide, chloroform, styrolene		18	1	(18)
	Petroleum fractions		0	2	(220)
		50 to 760 mm.	0		(151)
	Stannic chloride		30		(98)
	Blood fluids	Atm.	37.5	4	(105)
	Aqueous solutions.				
	Solute:				
	Acetone		25	3	(169)
	NaCl		0	2	(220)
	NH ₄ Cl, KCl, NaCl, MgCl ₂ , CaCl ₂ , BaCl ₂ , AlCl ₃ , FeCl ₃ , NH ₄ Br, NaBr, KBr, NaNO ₃ , KNO ₃ , Mg(NO ₃) ₂ , Ca(NO ₃) ₂ , Zn(NO ₃) ₂ , Al(NO ₃) ₃ , (NH ₄) ₂ SO ₄ , Na ₂ SO ₄ , K ₂ SO ₄ , MgSO ₄ , ZnSO ₄ , MnSO ₄ , NiSO ₄ , CoSO ₄ , FeSO ₄ , Al ₂ (SO ₄) ₃ , Cr(SO ₄) ₃ , Fe ₂ (SO ₄) ₃	Atm.	25	4	(198)
	Ba(OH) ₂ , Ca(OH) ₂ , NH ₄ OH, NaOH, KOH, Na ₂ SO ₄ , K ₂ SO ₄	Atm.	15	3	(23)
	Acidified sodium sulfate solution	Atm.	25	4	(158)

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPERATURE °C.	VALUE	REFERENCES
<i>C. Compound gases— Continued:</i> 21 Dimethyl ether	Acetone, methyl acetate	230 to 1000 mm.	25	4	(133)
	Benzene	90 to 1000 mm.	25	4	(133)
	Carbon tetrachloride	100 to 1000 mm.	25	4	(133)
	Chlorobenzene	10 to 1000 mm.	25	4	(133)
	Olive and sesame oils		17-37	3	(209)
22. Methyl chloride	Acetone, methyl acetate	230 to 1000 mm.	25	4	(133)
	Benzene	90 to 1000 mm.	25	4	(133)
	Carbon tetrachloride	100 to 1000 mm.	25	4	(133)
	Chlorobenzene	10 to 1000 mm.	25	4	(133)
	Olive and sesame oils		17-37	3	(209)
	Chloroform	200 to 900 mm.	25	4	(133)
23. Chloroethylene	Ethanol, ethylene dichloride, petroleum fractions	50 to 760 mm.	0		(151)
24. Fluoroethane	Water		14	2	(214)
	Ethanol, ethyl bromide, diethyl ether			1	(214)
25. Fluoroethylene	Ethanol, acetone		20	1	(301)
26 Carbon monoxide.	Water	Atm.	0-100	4	(340)
		Atm.	0-60	4	(339)
		Atm.	20-25	3	(147)
		Atm.	20	3	(49)
		Atm.	0-25	2	(37, 38, 39, 40)
	900 to 8200 mm.		20-25	2	(45)
		Atm.	20	2	(185)
		Atm.	25	3	(289)
		Atm.	20-25	3	(147)
		Atm.	20-25	3	(147)
	Ethanol	Atm.	20	3	(49)
		Atm.	25	3	(289)
		Atm.	0-25	2	(44)
		Atm.	0-25	2	(40)
		Atm.	25	3	(289)
	Glycerol	Atm.	20-25	3	(147)
	Amyl alcohol	Atm.	25	4	(46)
	Cyclohexanol	Atm.	25	3	(289)
	Acetic acid	Atm.	20-25	3	(147)
	Methyl acetate	Atm.	-80-+40	4	(133)
	Ethyl acetate, isobutyl acetate, amyl acetate	Atm.	20-25	3	(147)
	Benzene	Atm.	10-60	4	(133)
		Atm.	25	3	(289)
		Atm.	20-25	3	(147)
	Toluene, xylene	Atm.	20-25	3	(147)
	Chlorobenzene	Atm.	-40-+80	4	(133)

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPER- ATURE	VALUE	REFER- ENCES
			°C.		
<i>C. Compound gases—</i>					
Continued:					
26. Carbon mon- oxide . .	Carbon tetrachloride	Atm.	-20-+60	4	(133)
	Chloroform	Atm.	20-25	3	(147)
		Atm.	25	3	(289)
	Ethylene dichloride	Atm.	25	3	(289)
	Diethyl ether	Atm.	-80-+20	4	(133)
		Atm.	0-15	4	(50)
	Acetone	Atm.	-80-+40	4	(133)
		Atm.	25	3	(289)
		Atm.	20-25	3	(147)
	Carbon disulfide	Atm.	25	3	(289)
		Atm.	20-25	3	(147)
	Nitrobenzene	Atm.	25	3	(289)
		Atm.	20-25	3	(147)
	Aniline	Atm.	20-25	3	(147)
	Petroleum fractions	Atm.	20	3	(49)
		Atm.	10-20	3	(101)
					(88)
	Sulfuric acid	Atm.	20	3	(49)
	Blood	1 to 70 mm	39	1	(138)
	Aqueous solutions.				
	Solute				
	Alcohol	Atm.	20	2	(185)
	Acidified sodium sulfate	Atm.	25	4	(158)
	Cuprous ammonium car- bonate	150 to 2500 mm.	0-75	4	(107)
	Cuprous ammonium car- bonate and formate	12 to 370 mm.	0-80	4	(173)
	Non-aqueous solutions.	Atm.	25	3	(289)
	In benzene: phenanthrene, nitrobenzene, α -naphthol, β -naphthol, ethanol				
	In toluene: naphthalene, phenanthrene, aniline, α - naphthol, acetic acid				
	In acetone: phenanthrene, aniline, nitrobenzene, β - naphthol				
	In acetic acid: nitrobenzene, aniline, chloroform, ben- zene				
	In acetone: chloroform, car- bon disulfide				
	In methanol: glycerol, chlo- roform				
	In carbon disulfide: ethylene dichloride				
27. Carbon di- oxide.	Water	Atm.	25	4	(282)
		750 to 1400 mm.	25	4	(81)
		250 to 1000 mm.	25	4	(84)
		750 to 1400 mm.	25	4	(85)
		Atm.	25	4	(82)
		750 to 1400 mm.	25	4	(86)

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPER- ATURE °C.	VALUE	REFER- ENCES
<i>C. Compound gases— Continued: 27. Carbon di- oxide</i>	Water	260 to 760 mm.	25	4	(87)
		Atm.	0-25	4	(152)
		Atm.	25	4	(159)
		Atm.	18-36	4	(171)
		Atm.	25	4	(216)
		60 to 800 mm.	0-25	4	(215)
		Atm.	25	4	(230)
		Atm.	0	4	(247)
		Atm.	15	4	(284)
		Atm.	20	4	(320)
		Atm.	0-40	4	(201)
		Atm.	25	4	(61)
		Atm.	38	4	(326)
		Atm.	15	3	(112)
		Atm.	25	3	(100)
		Atm.	15	3	(48)
		Atm.	15-25	3	(147)
		Atm.	20	3	(49)
		520 to 720 mm.	0-20	2	(40)
		Atm.	0-20	2	(37, 38, 39, 40)
				2	(6)
		Atm.	0-60	2	(25)
		560 to 875 mm.	15	2	(279)
		Atm.	15-60	2	(275)
		4 atm.	0	2	(233)
		Atm.	20-25	2	(207)
		Atm.	15-21	2	(219)
		Atm.	8-30	2	(191)
		Atm.	0-40	1	(113)
		700 to 1300 mm.	15	1	(149)
		Relative pressures	11	1	(238)
		500 to 800 mm.	12	1	(36)
			0		(97)
			20		(343)
			17-20		(84)
		Atmospheric to very low	20		(35)
	Heavy water	Atm.	25	4	(61)
	Sea water	500 to 800 mm.	12	1	(36)
	Methanol	Atm.	18-36	4	(171)
		Atm.	15-20	3	(168)
	Ethanol	50 to 760 mm.	-78-59	3	(290)
		Atm.	15-25	3	(147)
		Atm.	18-36	4	(171)
		Atm.	15-25	3	(147)
		50 to 760 mm.	-78-59	3	(296)
			15-20	3	(168)
		Atm.	20	3	(49)
		Atm.	0-20	2	(40)
		Atm.	0-25	2	(44)
		Atm.	-67-45	2	(26)
	Propanol	Atm.	20-25	2	(207)
		Atm.	15-25	3	(147)

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPER- ATURE	VALUE	REFER- ENCES
			°C.		
<i>C. Compound gases—</i>					
Continued:					
27. Carbon di- oxide	Isobutyl alcohol	Atm.	15-25	3	(147)
	Amyl alcohol	Atm.	15-25	3	(147)
	Isoamyl alcohol	Atm.	18-36	4	(171)
	Cyclohexanol	Atm.	25	4	(46)
	Acetic acid	Atm.	18-36	4	(171)
		Atm.	15-25	3	(147)
	Propionic acid	Atm.	15-25	3	(147)
	Butyric acid	Atm.	15-25	3	(147)
	Formic acid	Atm.	7		(67)
	Acetic acid	Atm.	15		(97)
	Methyl acetate	Atm.	25	4	(130)
		Atm.	15-25	3	(147)
		50 to 760 mm.	-78--59	3	(296)
	Ethyl acetate	50 to 760 mm.	-78--59	3	(296)
	Amyl acetate	Atm.	18-36	4	(171)
		Atm.	15-25	3	(147)
	Amyl formate	Atm.	15-25	3	(147)
	Isobutyl acetate	Atm.	15-25	3	(147)
	Benzene	Atm.	5.5		(97)
			5.5		(98)
		Atm.	15-25	3	(147)
	Toluene	Atm.	15-25	3	(147)
	Chloroform	Atm.	18-36	4	(171)
		Atm.	0	4	(130)
		Atm.	15-25	3	(147)
		36 to 760 mm.	13	3	(329)
	Bromoform	Atm.	9		(97)
			9		(98)
	Carbon tetrachloride	Atm.	0-25	4	(130)
		Atm.	25	4	(131, 133)
		Atm.	15-25	3	(147)
	Ethyl chloride	Atm.	17.5	2	(307)
	Ethylene dichloride	Atm.	15-25	3	(147)
	Ethylene dibromide	Atm.	18-36	4	(171)
		Atm.	15-25	3	(147)
	Acetone	Atm.	10-25	4	(130)
		Atm.	18-36	4	(171)
		Atm.	15-25	3	(147)
		50 to 760 mm.	-78--59	3	(296)
	Acetophenone	Atm.	16		(97)
	Diethyl ether	Atm.	0	4	(130)
			-64+15	2	(307)
	Pyridine	Atm.	18-36	4	(171)
		Atm.	15-25	3	(147)
	Carbon disulfide	100 to 900 mm.	7-20	3	(328)
		Atm.	15-25	3	(147)
	Nitrobenzene	Atm.	15-25	3	(147)
			6		(98)
	p-Azoxypheetole	Atm.	145-170	3	(128)
	Chlorobenzene	Atm.	25	4	(130)
		Atm.	15-25	3	(147)
	Aniline	Atm.	18-36	4	(171)
		Atm.	15-25	3	(147)

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPERATURE °C.	VALUE	REFERENCES
<i>C. Compound gases— Continued. 27 Carbon dioxide</i>	Benzaldehyde	Atm.	18-36	4	(171)
		Atm.	15-25	3	(147)
	Glycerol, bromobenzene, iodobenzene, benzyl chloride, propylene bromide, amyl bromide, amyl chloride, isobutyl chloride, benzotrichloride, <i>o</i> -toluidine, <i>m</i> -toluidine, acetic anhydride, dichlorohydrin, eumene, eugenol	Atm.	15-25	3	(147)
	Sulfuric acid	Atm.	25	4	(201a)
		Atm.	20	3	(49)
	Petroleum fractions	Atm.	20	3	(49)
		Atm.	2-25	2	(170)
		Atm.	10-20	2	(101)
		Atm.	20-50		(268)
					(88)
	Cottonseed, herring, olive, linseed, maize, and cod-liver oils	Atm.	20-50		(268)
	Cottonseed oil, corn oil, lard		23-45		(327)
	Blood	Atm.	38	4	(326)
	Beer	750 to 1400 mm	25	4	(85)
	Aqueous solutions:				
	Solute				
	Ethanol	Atm.	15	4	(284)
		750 to 1400 mm.	25	4	(85)
		Atm.	0-25	4	(152)
		Atm.	15-21	2	(219)
		0			(310)
	Dextrose, levulose, sucrose	Atm.	15	4	(284)
	Glycerol, acetone, urea	Atm.	0-25	4	(152)
	Glycerol, chloral hydrate	Atm.	15	3	(112)
	Sucrose, chloral hydrate, KCl, Fe(NH ₄) ₂ (SO ₄) ₂ , NH ₄ Cl, BaCl ₂	750 to 1400 mm.	25	4	(86)
	Propanol, acetic acid, acetamide, antipyrine, urea, thiourea, urethan, catechol, resorcinol, quinol, pyrogallol, glycine, mannitol, dextrose, sucrose	Atm.	20	4	(320)
	Ethanol, acetone, urea, glycerol		0		(310)
	KCl	750 to 1400 mm.	25	4	(81)
	NaCl, KCl, Na ₂ HPO ₄ , K ₂ HPO ₄ , sodium lactate, potassium lactate	Atm.	38	4	(326)
	H ₂ SO ₄ , NaCl, Na ₂ SO ₄ , Na ₂ PO ₄ , CaCl ₂ , MgCl ₂ , ZnCl ₂ , AlCl ₃ , Al ₂ (SO ₄) ₃	Atm.	25	4	(159)
	HNO ₃ , HCl, H ₂ SO ₄ , CsCl, KNO ₃ , KI, RbCl, KBr, KCl	Atm.	15-25	3	(100)

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPER- ATURE	VALUE	REFER- ENCES
<i>C. Compound gases— Continued: 27. Carbon di- oxide</i>			°C.		
	Sucrose, LiCl, NaCl, KCl, KBr, KI, KNO ₃ , H ₂ SO ₄ , MgSO ₄ , CuSO ₄ , ZnSO ₄ , (NH ₄) ₂ SO ₄ , KHSO ₄ , KHSO ₄ , KH ₂ AsO ₄ , KH ₂ PO ₄ , K ₂ HAsO ₄ , K ₂ HPO ₄ , Na ₂ PO ₄ , Na ₄ P ₂ O ₇ , Na ₂ B ₄ O ₇ , NaCl, NaNO ₃ , Na ₂ SO ₄ , KCl, KNO ₃ , Mg(NO ₃) ₂ , MgSO ₄	Atm.	15	3	(48)
	H ₂ SO ₄ , HClO ₄	Atm.	25	4	(201a)
	Citric, tartaric, metaphos- phoric acids; NaNO ₃ , NaBr, Na ₂ SO ₄ , LiCl, Mg- Cl ₂ , MgSO ₄ , Ca(NO ₃) ₂ , CaCl ₂ , Co(NO ₃) ₂ , K ₄ Fe(CN) ₆ , ZnSO ₄ , Zn(NO ₃) ₂ , Pb(NO ₃) ₂ , NH ₄ Cl, (NH ₄) ₂ SO ₄ , KCl, KBr, KI, KCNS, NaClO ₃	Atm.	0-40	4	(201)
	NaCl, KCl, NH ₄ Cl	Atm.	8-22	2	(191)
	CaCl ₂ , SrCl ₂ , BaCl ₂	Atm.	8-30	2	(191)
	NaCl	Atm.	0-60	2	(25)
	(NH ₄) ₂ CO ₃	3 to 12 mm	25	3	(33)
	MgSO ₄ , CaSO ₄	500 to 800 mm.	12	1	(86)
	NaCl, CaCl ₂		20		(343)
	NaCl				(6)
	Acidified sodium sulfate so- lution	Atm.	25	4	(158)
	Sugar liquors				(195)
	Ternary solutions: Dextrose-ethanol-water	Atm.	15	4	(284)
	Sucrose-ethanol-water				
	Non-aqueous solutions: Methanol and ethanol solu- tions of LiCl, LiBr, LiI, NaCl, NaBr, NaI		15-20	3	(168)
	Acetic acid-carbon tetrachlo- ride and carbon disulfide- ethylene dichloride solu- tions	Atm.	15	3	(48)
28. Carbonyl sulfide	Water	Atm.	13.5	2	(116)
			20	2	(299)
	Ethanol		20	1	(299)
	Toluene		20	1	(299)
			-14	1	(230)
	Carbon disulfide		20	1	(299)
	Aqueous sodium chloride solu- tion		20	1	(299)

TABLE 1—*Continued*

GAS	SOLVENT	PRESSURE	TEMPERATURE	VALUE	REFERENCES
			°C.		
<i>C. Compound gases—</i> Continued:					
29 Carbonyl chloride.	Ethanol, acetic acid, benzene	Atm.	20	2	(11)
	Toluene	Atm.	12-31	3	(7)
		Atm.	20	2	(11)
	Xylene	Atm.	12-31	3	(7)
	Chloroform, carbon tetrachloride	Atm.	20	2	(11)
	Chlorobenzene, acetylene tetrachloride, creosote	Atm.	12-31	3	(7)
	Petroleum fractions	Atm.	12-31	3	(7)
		Atm.	20	2	(11)
30. Cyanogen.	Water and aqueous hydrochloric acid solution		18	1	(223)
31 Hydrogen cyanide	Water	1 to 7 mm (?)	25	2	(179)
32 Silane	Cyclohexanol	Atm.	25	4	(46)
33 Ammonia	Water	6 to 500 mm.	0-60	4	(241)
		1 to 100 mm.	0-25	4	(215)
		750 to 4000 mm.	0-40	4	(226)
		0.024 to 1.6 mm.	0	4	(154)
		8 to 60 mm.	60	4	(66)
		6 to 14 mm.	25	4	(99)
		Distillation under atmospheric pressure		4	(42)
		20 to 1800 mm.	0-61	3	(239)
		200 to 1000 mm.	20-60	3	(240)
		1 to 15 mm.	25	3	(183)
		200 to 2000 mm.	0-100	3	(287)
		About 2 to 70 mm.	20	3	(184)
		10 to 2000 mm.	0	3	(260)
		Atm.	0-56	3	(260)
		13 mm.	25	3	(1)
		Atm.	0-29	2	(254)
		Atm.	0-20	1	(40)
		Relative pressures	16	1	(238)
	Methanol	Atm.	0-28	3	(182)
	Ethanol	Atm.	0-28	3	(182)
			0	1	(9)
	Cyclohexanol	Atm.	25	4	(46)
	Quinoline	Relative pressures	16	1	(238)
	Benzene, toluene, hexane, octane, dodecane, cetane, carbon tetrachloride, chloroform, ethylene dichloride, chlorobenzene, bromobenzene, benzyl chloride	Atm.	20	4	(15)

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPERATURE	VALUE	REFERENCES
C Compound gases— Continued: 33. Ammonia ..			°C.		
	Aqueous solutions				
	Solute:				
	NaOH, NH_4Cl , NH_4NO_3 , NH_4I , NH_4CNS , $(\text{NH}_4)_2\text{SO}_4$, $(\text{NH}_4)_2\text{C}_2\text{O}_4$, $(\text{NH}_4)_2\text{C}_4\text{H}_4\text{O}_4$, KCl , NaCl , BaCl_2 , CaCl_2 , SrCl_2 , MgCl_2 , AgCl , CuCl , CuSO_4 , ZnSO_4 , CdSO_4	6 to 14 mm.	25	4	(99)
	CuSO_4	1 to 15 mm.	25	3	(183)
	Urea, mannitol, K_2SO_4 , NH_4Cl , CuSO_4	200 to 1000 mm.	20–60	3	(240)
	NH_4CNS	Up to 2 atm.	10–30	3	(91)
	KCl , KBr , KI , KOH , KF , NaCl , NaBr , NaI , NaOH , LiCl , LiBr , LiI , LiOH , KNO_3 , KNO_2 , KCN , KCNS , KBO_3 , K_2SO_4 , K_2CO_3 , K_2CrO_4 , $\text{K}_2\text{C}_2\text{O}_4$, CH_3COOK , K_2HPO_4 , Na_2S , KClO_4 , KBrO_3 , KIO	13 mm.	25	3	(1)
	$(\text{NH}_4)_2\text{CO}_3$	8 to 23 mm	25	3	(33)
	NH_4Cl , NaNO_2 , NH_4NO_2 , KOH , NaOH , $\text{Ca}(\text{NO}_3)_2$	Atm	0–29	2	(254)
	KOH , NaOH , K_2CO_3 , CH_3COOK , $(\text{COOK})_2$, KCl , Na_2CO_3 , CH_3COONa , HCOONa , NaCl , BaCl_2 , SrCl_2 , CaCl_2 , LiCl , NH_4Cl , $(\text{NH}_4)_2\text{SO}_4$, K_2SO_4 , KCl , KNO_3 , KBr , KI , $\text{Cd}(\text{NO}_3)_2$, $\text{Zn}(\text{NO}_3)_2$, $(\text{HCOO})_2\text{Ba}$, $(\text{CH}_3\text{COO})_2\text{Ba}$, NiCl_2 , $\text{Cu}(\text{NO}_3)_2$, AgNO_3 , NiSO_4 , CuCl_2 , CuSO_4 , $(\text{CH}_3\text{COO})_2\text{Cu}$	About 60 mm	60		(165, 166)
	Distribution data:				
	Water-ether		20	4	(184)
	Water-chloroform		25	4	(16)
			20	3	(64)
	Chloroform-aqueous solutions:				
	Solute: CuSO_4 , CuCl_2 , CdI_2 , NiSO_4 , Na_2SO_4 , CuO , ZnSO_4		20	3	(64)
34. Methylamine	Water	8 to 60 mm.	60	4	(66)
		2 to 17 mm.	25	4	(80)
			12.5	1	(345)
	Distribution data:				
	Water-ether		25	4	(290)
	Water-xylene		25	4	(290)

TABLE 1—*Continued*

GAS	SOLVENT	PRESSURE	TEMPERATURE °C.	VALUE	REFERENCES
<i>C. Compound gases—</i>					
<i>Continued:</i>					
35. Dimethyl-amine	Water	3 to 26 mm.	25	4	(80)
			25	4	(290)
36. Trimethyl-amine	Water	17 to 133 mm. 35 to 60 mm. 9 to 13 mm. 7 to 12 mm. 8 to 13 mm. 16 mm. 1 to 25 mm. 22 to 42 mm. 28 to 33 mm. 5 to 9 mm. 50 to 85 mm. 52 to 77 mm. 70 to 120 mm. 30 to 50 mm. 35 to 50 mm. 35 to 56 mm. 85 mm. 65 to 95 mm. 50 to 60 mm. 40 mm.	25	4	(80)
			16-22	2	(174)
			25	3	(108)
37. Ethylamine..	Water	8 to 60 mm. 2 to 18 mm.	60	4	(66)
			25	4	(62)
			25	4	(290)
38. Diethyl-amine.	Water	4 to 30 mm.	25	4	(62)
39. Triethyl-amine.	Water	5 to 30 mm. 15 to 170 mm. 2 mm. 8 to 14 mm.	25	4	(62)
			6-50	3	(174)
			25	3	(108)
			25	3	(108)
40. Propylamine..	Water	8 to 60 mm.	60	4	(66)
41. Nitrous oxide	Water	750 to 1400 mm. 250 to 1000 mm. Atm. Atm. Atm. Atm. Atm. Atm. Atm. Atm. Atm.	25	4	(81)
			25	4	(83)
			0-40	4	(201)
			25	4	(230)
			25	4	(198)
			18-36	4	(171)
			25	3	(100)
			20	3	(157)
			5-20	3	(262)
			15-80	2	(275)
			8-22	2	(104)

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPER- ATURE	VALUE	REFER- ENCES
			°C.		
<i>C. Compound gases—</i>					
Continued:					
41. Nitrous oxide.	Water	Atm.	0-25	2	(40)
		Atm.	0		(97)
					(288)
	Ethanol	Atm.	18-36	4	(171)
		Atm.	0-25	2	(40)
		Atm.	0-25	2	(44)
		Atm.	16-18	2	(186)
	Benzene	Atm.	10-40	4	(130, 133)
		Atm.	5.5		(97)
	Acetone	Atm.	0-40	4	(130, 133)
		Atm.	18-36	4	(171)
	Acetic acid	Atm.	18-36	4	(171)
		Atm.	15		(97)
	Methyl alcohol	Atm.	18-36	4	(171)
	Isoamyl alcohol	Atm.	18-36	4	(171)
	Cyclohexanol	Atm.	25	3	(46)
	Formic acid	Atm.	7		(97)
	Methyl acetate	Atm.	10-40	4	(130, 133)
	Amyl acetate	Atm.	18-36	4	(171)
	Chloroform	Atm.	18-36	4	(171)
	Carbon tetrachloride	Atm.	10-40	4	(130, 133)
	Bromoform	Atm.	7		(97)
	Ethylene dibromide	Atm.	18-36	4	(171)
	Chlorobenzene	Atm.	10-55	4	(130, 133)
	Acetophenone	Atm.	16		(97)
	Pyridine	Atm.	18-36	4	(171)
	Benzaldehyde	Atm.	18-36	4	(171)
	Aniline	Atm.	18-36	4	(171)
	Petroleum fractions	Atm.	10-20	2	(101)
	Olive and sesame oils	Atm.	17-37	3	(209)
	Aqueous solutions:				
	Solute:				
	Propionic acid, chloral hy- drate	Atm.	20	3	(157)
	Urea, oxalic acid, glycerol		5-25	3	(262)
	NH ₄ Cl, KCl, CaCl ₂ , NaCl, BaCl ₂ , NH ₄ Br, KBr, NaBr, NH ₄ NO ₃ , KNO ₃ , NaNO ₃ , Mg(NO ₃) ₂ , Ca(NO ₃) ₂ , Zn(NO ₃) ₂ , Cd(NO ₃) ₂ , Cu(NO ₃) ₂ , Al(NO ₃) ₃ , (NH ₄) ₂ SO ₄ , K ₂ SO ₄ , Na ₂ SO ₄ , MgSO ₄ , ZnSO ₄ , MnSO ₄ , FeSO ₄ , CoSO ₄ , NiSO ₄ , Al ₂ (SO ₄) ₃ , Fe ₂ (SO ₄) ₃ , Cr ₂ (SO ₄) ₃ , KIO ₃ , Na ₂ HPO ₄ , Na ₂ PO ₄	Atm.	25	4	(198)
	NaCl, Na ₂ SO ₄ , KCl, KNO ₃ , Mg(NO ₃) ₂ , MgSO ₄	Atm.	0-40	4	(201)
	KNO ₃ , NaNO ₃	Atm.	20	3	(157)
	H ₃ PO ₄ , NaCl		5-25	3	(262)
	LiCl, NaCl, KCl, Na ₂ SO ₄ , K ₂ SO ₄ , Li ₂ SO ₄ , CaCl ₂ , SrCl ₂ , MgSO ₄	Atm.	8-22	3	(104)

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPER- ATURE °C.	VALUE	REFER- ENCES
<i>C. Compound gases—</i>					
Continued:					
41 Nitrous oxide	HNO ₃ , HCl, H ₂ SO ₄ , CsCl, KNO ₃ , KI, RbCl, KBr, KCl, LiCl, NH ₄ Cl, KOH H ₂ SO ₄ , FeSO ₄ , NaOH, pyrogallol (alkaline) Acidified sodium sulfate so- lution Blood fluids	Atm	15-25	3	(100)
			16-18	2	(186)
		Atm.	25	4	(158)
		Atm.	37.5	4	(230) (285)
42 Nitric oxide	Water	Atm.	0-100	3	(340)
		Atm.	0-60	3	(339)
			20	2	(319)
		250 to 2000 mm.	0-16	2	(200) (98)
	Ethanol	Atm.	0-25	2	(40)
		Atm.	0-25	2	(44)
	Benzene	520 to 1000 mm.	9-35	3	(156) (98)
			5		
	Carbon tetrachloride	450 to 1000 mm.	9-35	3	(156)
	Nitrobenzene	450 to 1000 mm.	20-90	3	(156) (98)
			5		
	Bromoform		8		(98)
	Cyclohexane		6		(98)
	Aqueous solutions:				
	Solute:				
	H ₂ SO ₄	Atm.	18	3	(314)
		Atm.	18	2	(187)
			0	2	(199)
				1	(197)
	FeSO ₄ , FeCl ₂ NiSO ₄ , CoSO ₄ , MnCl ₂ , fer- rous salt	250 to 2000 mm.	0-16	2	(200)
		550 to 700 mm.	20	2	(137)
	Ferrous salts Ethanol solution of ferrous chloride			1	(308)
		700 to 2000 mm.	2-28	2	(200)
43. Phosphine	Water		15	1	(73)
	Cyclohexanol	Atm.	25	4	(46)
44. Methyl- phosphine	Ethanol, ether		0	1	(126)
45. Arsine	Water	150 to 760 mm.	20	4	(99a)
		200 mm.	0-25	4	(146)
		100 to 360 mm.	20	4	(343a)
46. Stibine.	Water		Room	2	(298)
				1	(143)
	Ethanol, benzene Carbon disulfide		Room	2	(298)
			0	2	(298)
47. Hydrogen sulfide	Water	270 to 3500 mm.	5-60	4	(344)
		Atm.	0	4	(247)
		Atm.	25	4	(148)

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPERATURE °C.	VALUE	REFERENCES
C. Compound gases— Continued: 47 Hydrogen sulfide	Water	Atm.	25	4	(244)
		Atm.	0-25	4	(152)
		Atm.	0-40	3	(37, 38, 39, 40)
		Relative pressures	12	1	(238)
			0		(97)
	Ethanol	Atm.	0-25	3	(40)
		Atm.	0-25	3	(44)
	Glycerol		25	3	(205)
	Ether	740 mm.	26	3	(232)
	Aniline	100 to 1200 mm.	22	4	(10)
	Pyridine	750 mm.	22	1	(259)
	Benzene, hexane, cyclohexane, octane, dodecane, cetane, carbon tetrachloride, chloro- form, chlorobenzene, bromo- benzene, toluene, ethylene dichloride, trichloroethylene, tetrachloroethylene, penta- chloroethane, ethyl bromide, bromoform, <i>s</i> -tetrachloro- ethane, <i>s</i> -tetrabromoethane	Atm.	20	4	(15)
	Benzene		5.5		(97)
	Bromoform		7		(97)
	Formic acid		7		(97)
	Acetic acid		15		(97)
	Acetophenone		16		(97)
	Sulfur		440	1	(236)
	Aqueous solutions:				
	Solute:				
	HCl	Atm.	25	4	(148)
	HI	Atm.	25	4	(244)
	NaHS		15-45	2	(102)
	Ethanol	Atm.	0-25	4	(152)
			0		(310)
	Glycerol	Atm.	0-25	4	(152)
			0		(310)
	Acetone	Atm.	0-25	4	(152)
			0		(310)
	Urea	Atm.	0-25	4	(152)
			0		(310)
	HCl, NaCl, NH ₄ Cl, NaNO ₃ , KNO ₃ , NH ₄ NO ₃ , NaBr, KBr, NH ₄ Br, KI, CH ₃ COONH ₄ , H ₂ SO ₄ , Na ₂ SO ₄ , K ₂ SO ₄ , (NH ₄) ₂ SO ₄		25	3	(205)
48. Sulfur di- oxide.	Water	250 to 2500 mm.	10-27	4	(189)
		100 to 1500 mm.	20-110	4	(22)
		30 to 800 mm.	0-25	4	(215)
		Atm.	10-90	4	(135)
		50 to 1100 mm.	5-80	4	(291)

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPERATURE	VALUE	REFERENCES
°C.					
<i>C. Compound gases—</i> Continued. 48 Sulfur dioxide	Water	Atm.	25-35	4	(92)
		78 mm.	25	4	(92)
		0 to 180 mm.	15-25	4	(21)
		20 to 2000 mm.	7-50	3	(287)
		0 to 2800 mm	0-12	3	(13)
		Atm	0-40	2	(38, 39, 40)
		Relative pressures	12	1	(238)
		Atm.	0-100		(229)
	Methanol	Atm.	0-28	3	(182)
	Ethanol	Atm.	0-26	3	(182)
		Atm.	0-40	2	(40)
		Atm.	0-25	4	(133)
	Methyl acetate, acetone	200 to 1000 mm.	25	4	(133)
	Benzene	100 to 1000 mm.	25	4	(133)
		Atm.	25	4	(134)
		Atm.	30-60	4	(181)
	Toluene	Atm.	20-60	4	(181)
	Nitrobenzene, <i>o</i> -nitrotoluene	Atm.	15-60	4	(181)
	Carbon tetrachloride	Atm.	25-40	4	(130)
		100 to 950 mm.	25	4	(133)
	Chlorobenzene	10 to 1050 mm.	25	4	(133)
	Acetic anhydride	Atm	-5-+30	4	(181)
	Camphor	700 mm.	4-24	2	(271)
	Sulfuric acid	Atm.	20	3	(214)
	Aqueous solutions.				
	Solute:				
	H ₂ SO ₄	Atm.	20	3	(214)
		Atm	10-15	2	(72)
	KCl, Na ₂ SO ₄	Atm.	10-90	4	(135)
	Ca(HSO ₃) ₂	0 to 180 mm.	15-25	4	(21)
	Ca(HSO ₃) ₂ , Mg(HSO ₃) ₂	50 to 1100 mm	5-60	4	(291)
	KI, KBr, KCl, KCNS, NH ₄ NO ₃ , KNO ₃ , (NH ₄) ₂ SO ₄ , CdI ₂ , Na ₂ SO ₄ , CdBr ₂ , CdCl ₂ , CdSO ₄	Atm.	25-35	4	(92)
	KI, KCNS, KBr, KCl, KNO ₃ , (NH ₄) ₂ SO ₄	78 mm.	25	4	(92)
	Acidified sodium sulfate solution	Atm.	25	4	(158)
49. Hydrogen selenide	Water, hydriodic acid Selenium	Atm.	15-35	4	(203)
			580	1	(236)
50. Hydrogen chloride	Water	0.04 to 580 mm.	50	4	(346)
		0.01 to 4 mm.	25-30	4	(12)
		0.001 to 0.1 mm.	30	3	(95)
		80 to 1300 mm.	0-100	3	(260)
		0 to atmospheric	30	3	(67)
			-12-0	1	(19)
		Relative pressures	11	1	(228)

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPER- ATURE °C.	VALUE	REFER- ENCES
C. Compound gases— Continued: 50. Hydrogen chloride ..	Methanol	Atm.	0-32	3	(182)
	Ethanol	Atm.	0-32	3	(182)
	Chloroform		10	2	(384)
	Benzene, hexane, cyclohexane, octane, dodecane, cetane, carbon tetrachloride, chloro- form, bromoform, chloro- benzene, bromobenzene, tol- uene, ethylene dichloride, trichloroethylene, tetrachlo- roethylene, pentachloro- ethane, ethyl bromide, <i>s</i> -tetrachloroethane, <i>s</i> -tetra- bromoethane, benzotrichlo- ride, benzyl chloride	Atm.	20	4	(15)
	1,1,2-Trichloroethane, penta- chloroethane	500 to 700 mm.	12-20		(110)
	Ethylene dichloride, ethylene dibromide, acetylene tetra- chloride, carbon tetrachloride	300 to 800 mm.	15-25		(109)
	Diethyl ether		-9-+30	3	(273)
	Isoamyl ether	Atm.	0-25	3	(237)
	Aqueous solutions: Solute:				
	Ethanol	0.04 to 5 mm.	25	3	(145)
	Sulfuric acid		17-70		(56) (60)
51. Hydrogen bromide	Water	0.001 to 0.1 mm.	25 10	4 1	(12) (19)
	Benzene	8 to 630 mm.	30-50		(147a)
52. Hydrogen iodide	Water	0.0005 to 0.1 mm.	25 10	4 1	(12) (19)
D. Radioactive gases:† 1. Radium ema- nation	Water		0-100		(302)
					(315)
			0-80		(272)
			0-40		(29)
			15-30		(124)
			0-80		(127)
			0-91		(151)
			0-100		(208)
			0-15		(252)
	Sea water		14		(29)
	Methanol		15-30		(124)
	Ethanol		-18-+18		(252)
			-18-+18		(208)
			-18-+50		(272)
			14		(29) (315)

† The pressures are not given directly. The methods used were all similar, and there is relatively little basis for evaluating the results, except the temperature control.

TABLE 1—Continued

GAS	SOLVENT	PRESSURE	TEMPER- ATURE	VALUE	REFER- ENCES
<i>D. Radioactive gases†</i> —Continued: 1 Radium emanation			°C.		
	Propanol, butanol, formic acid, acetic acid, propionic acid, butyric acid		15-30		(124)
	Amyl alcohol		14		(29)
	Glycerol		18		(252)
	Ethyl acetate		-18-+18		(208)
			-18-+18		(252)
			-18-+60		(272)
	Benzene		6-73		(302)
			18		(252)
			18		(272)
	Toluene		-18-+18		(208)
			14		(29)
			-18-+60		(272)
			-18-+18		(252)
	Xylene		-18-+18		(252)
			-20-+70		(208)
	Hexane		-18-+30		(272)
			-18-+18		(252)
	Cyclohexane		-18-+18		(252)
	Chloroform		-18-+18		(208)
			-18-+18		(252)
			-20-+50		(272)
	Aniline		-18-+18		(252)
					(315)
			0-18		(272)
	Acetone		-18-+18		(208)
			-20-+40		(272)
			-18-+18		(252)
	Carbon disulfide		-18-+40		(272)
			-18-+18		(208)
			-18-+18		(252)
	Diethyl ether		-18-+18		(208)
			-18-+18		(252)
			-18-+30		(272)
	Petroleum fractions		-18-+18		(252)
			-21-+60		(127)
					(315)
	Aqueous solutions. Solute:				
	CuSO ₄				(315)
	NaCl, Ba(NO ₃) ₂ , NH ₄ NO ₃ , urea		5-30		(162)
	Ethanol, sucrose, KCl, NaCl, NH ₄ Cl, Pb(NO ₃) ₂ , AgNO ₃ , HgCl ₂ , ZnSO ₄ , CuSO ₄ , FeSO ₄ , KMnO ₄ , K ₄ Fe(CN) ₆		18		(160)
2. Thorium emanation	Water		Room		(155)
	Ethanol				(28)

TABLE 1—*Concluded*

GAS	SOLVENT	PRESSURE	TEMPERATURE °C.	VALUE	REFERENCES
D. Radioactive gases† —Continued: 2. Thorium emanation. . .	Petroleum fractions		Room		(155) (28) (28)
	Aqueous solutions of H ₂ SO ₄ , CuSO ₄ , CaCl ₂				
3. Actinium emanation . .	Water, acetone, benzene, ethanol, amyl alcohol, benzaldehyde, toluene, carbon disulfide, petroleum, sulfuric acid, aqueous KCl solution		Room		(119)

† The pressures are not given directly. The methods used were all similar, and there is relatively little basis for evaluating the results, except the temperature control.

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